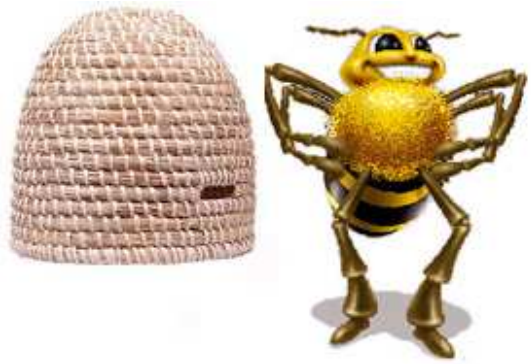
The Propolis Book

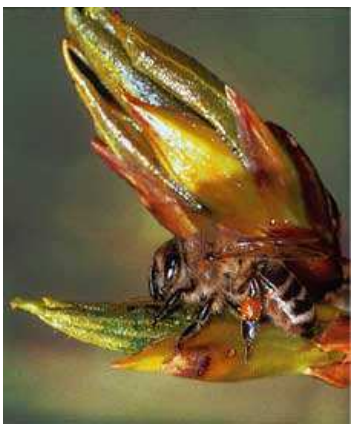


Other bees, like soldiers, armed in their stings,
Make boot upon the summer's velvet buds,
Which pillage they with merry march bring home.

Shakespeare, King Henry 5th

It seems that Shakespeare also knew that specialized bees gather propolis in the buds.

The word propolis originates from Greek: «pro» = in front, «polis» = city. The meaning „ in front of the city„ suits well the protecting role of propolis for the bee colony. The Greek world propolis means also to glue and describes also the role of propolis to cement openings of the bee hive. Another name of propolis is bee glue.



I would appreciate your feedback at info@bee-hexagon.net

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Propolis: Origin, Production, Compostion

Stefan Bogdanov and Vassya Bankova

PROPOLIS IN HISTORY

The word propolis originates from Greek: «pro» = in front, «polis» = city. The meaning „in front of the city suits well the protecting role of propolis for the bee colony. The Greek world propolis means also to glue and describes also the role of propolis to cement openings of the bee hive. Another name of propolis is bee glue.

Propolis was already known in ancient Egypt, where it was probably used as an adhesive. Propolis was mentioned by the Greek philosopher Aristoteles. In his *Historia animalium* it was reffered to a substance which the bees smeared at the hive entrance and used as cure for bruises and sores, Crane, p. 550 of [20].

The Greeks used propolis as the principal ingredient of an exquisite perfume called “polyanthus”, which combined propolis, olibanum styrax and aromatic herbs, while the ancient Jews considered “tsori” or propolis as a medicine.

The Roman scholar Plinius (23-79 A.D) postulated, that it originates in the buds of different trees like willow, poplar, elm, reed and other plants. He knew of the use of propolis as a glue in the hive and about its medicinal properties and described them in his 35 volumes Natural History. He says “*Current physicians use propolis as a medicine because it extracts stings and all substances embedded in the flesh, reduces swelling, softens indurations, soothes pain of sinews and heals sores when it appears hopeless for them to mend.* [81].

The Greek doctor Discorides, 1st century AD, thought that it came from *Styrax: the yellow bee glue that is of a sweet scent and resembling styrax is to be chosen and which is soft and easy to spread after the fashion of mastic. It is extremely warm and attractive and is good for the drawing out of thorns and splinters. And being suffimigated it doth help old coughs and being applied it doth take away the lichens”* [28].

The Arabs knew probably also about propolis. Doctor Avicenna speaks of two different kinds of wax: clean wax and black wax, the latter being probably propolis. He says: „*by its strong smell it makes you sneeze*“ and „*has the characteristics to eliminating the spikes of the bolts and the stakes. It also rarefies cleans and soaks.* [28].

In Europe it is mentioned in the herbal literatures. Other healers in the many centuries that followed also praised the use of bee products for healing. In John Gerard's famous herbal book, The History of Plants (1597), reference is also made to the use of "*the resin or clammy substance of the black poplar tree buds*" for healing ointments [28]. Nicholas Culpepper's famous Complete Herbal (1653), under the heading of "The Poplar Tree" states that "*the ointment called propolis is singularly good for all heat and inflammations in many parts of the body and cools the heat of the wounds*" [28]. In Green's Universal Herbal (1824), under *Populus nigra* (Black Poplar Tree), it is said that "*the young leaves are an excellent ingredient for poultices for hard and painful swellings. The buds of both this and the white poplar smell very pleasantly in the spring. Being pressed between the fingers, yield a balsamic resinous substance (propolis), which smells like storax. A drachma of this tincture in broth is administered in internal ulcers and excoriations and is said to have removed obstinate or abnormal discharges from the intestines"*

Although the main use of propolis was medical, it was used as a constituent of violin varnish. Violin builders like Stradivari, Amati and others used propolis as a constituent of their violin varnish p. 550 of [21],

Georgie is the origin of the propolis gathering Caucasian bees. In the medicine book “The Carbadini”, published in the 13th century, the author suggests that propolis is good against dental decay[28].

At the beginning of the 20th century a hypothesis was prevailing, that propolis is a digestion product of bee pollen [44]. In 1928 the German scientist Rösch, on the ground of meticolous observations, confirmed the hypothesis of Plinius that propolis originates from the buds of plants [82]. The Russian researcher Popravko proved this theory by comparing the composition of buds resin and the propolis [73, 74].
Propolis research and use in history until recent times has been reviewed[43]

BEES COLLECT PROPOLIS

Bees gather resinous exudates originating mainly from buds, but also from leaves, branches and barks. Recently, this topic, especially the role of propolis in bee health has been reviewed[92]. Indeed the principal role of propolis

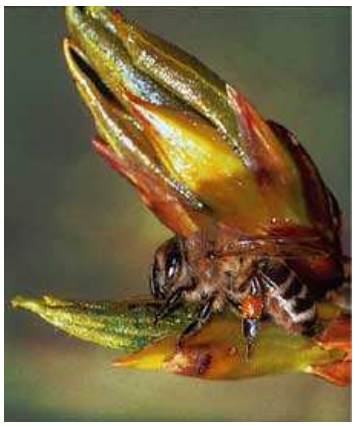
is to maintain an antiseptic environment in the bee hive and to enable the bee colony health. It promotes the social immunity of bees and helps them to fight infections. At present research is carried out to investigate the possible role of propolis constituents for maintaining bee colony health[56].

Propolis is gathered mostly from *Apis mellifica* bees. *A .mellifica Caucasica* is most industrious from all *A. mellifica* races. The Asian bees *Apis florea* and *Apis cerana* do not gather propolis. The tropical stingless bee species also gather propolis and incorporate it in wax to make cerumen. In temperate zones propolis is gathered in late summer and in autumn, when bees prepare for wintering.

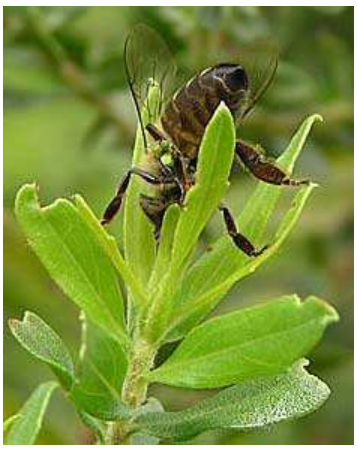
Meyer (1956) [55] and Morse (1975) [57] described in detail how bees gather propolis. According to these researchers only a few of the workers, not older than 15 days old, are specialised in propolis foraging. Bees gather propolis during the warm time of the day, when the glue is soft. Bees grab the soft glue from the bud and pull it out. Propolis is carrried to the hive like pollen in the form of a load, which contains also secretion products from the mandibular gland. One forager gathers into the hive about 10 mg propolis per flight. If it is assumed that an average bee colony gathers about 100 g propolis per year, then about 100‘000 foraging flights are needed for this purpose.

On the average, one colony gathers about 50-150 g propolis per year but the propolis specialists, the Caucasian bees can gather 250 to 1000 g propolis per year [89]

Bees cover the walls of the hive and mix it with wax for the comb construction to increase its strength. Killed intruders like snails, mice etc. are mummified with propolis. As propolis has strong antibacterial and fungicide properties they create a highly hygienic environment. Also, bees make a propolis „door matt“ on the hive entrance, so that every bee has to step on propolis before entering and leaving the hive. Bees use propolis for the disinfection of the hive as. Although propolis has different advantages for the bees, it has a practical disadvantage for beekeepers: Because of the gluey nature the frames often stick together and can be pulled out of the hive with difficulty.



Bee gathers propolis from poplar bud
Photo: courtesy Gilles Ratia



Bee gathers propolis from Baccharis
Unknown source from Internet

PROPOLIS ORIGIN

The propolis origin is determined by comparative chemical analysis of propolis and the glue from the botanical source. Some researchers use pollen analysis to determine propolis origin, however this method is not acceptable as pollen collected by the bees for their nutrition needs can be mixed with propolis in the hive.

Propolis sources in the temperate zone

Popravko (1970) from Russia was the first to present chemical evidence of the propolis botanical origin, analyzed flavonoid composition of propolis and comparing it with that of poplar and birch bud exudates. Many other publications followed and now it is generally accepted and chemically proved that in temperate zones the bud exudates of *Populus* species and their hybrids are the main source of bee glue. This is true for Europe, North America and the temperate part of Asia [3, 9, 29, 35, 69]

Meditaranian propolis produced in Malta seems to originate from cypress[72]. In Russia however, and especially in its northern parts, besides poplar, which seems to provide mainly propolis also birch buds

(*Betula verrucosa, Betula pubescens, Betula litwinowii*) and aspen can supply bees with the propolis, [34, 75]
In China besides the main source poplar bees also use pines, cypress, willow and sumacs[45]
In Turkey poplar seems to be the main origin, but also [69, 70, 90, 91, 101-103] but bees can also gather it from other sources as pine trees, eucalyptus and castanea[37, 91].

Plant sources of tropical propolis

In tropical regions there are no poplars and birches and obviously bees have to find new plant sources of bee glue.

The most popular tropical propolis type, the green Brazilian propolis, originates from the leaves of *Baccharis dracunculifolia* [15, 95]

The so called red propolis is gathered by bees in Cuba, Mexico and Brazil from *Dalbergia* species is characterized by the presence of isoflavonoids.[26, 98]

Another tropical propolis type is the one originating from resin exuded by the flowers of different *Clusia* species found in Cuba and Venezuela. Its main constituents are prenylated benzophenones. [30, 99]

In tropical islands in the Pacific Ocean (Taiwan, Okinawa, Indonesia), there is a specific propolis type, designated sometimes as “Pacific propolis”. It contains prenylated flavanones (propolins) as major constituents [18, 33, 41] and its plant source is the rein on the fruits of the tropical tree *Macaranga tanarius* [42]

Bees in Venezuela gather propolis also on the poplar species of *Aigeiros* (Salicaceae) [11]

Propolis plant sources in the Subtropics

One of the most important Subtropical propolis types turned out to be the so called Mediterranean propolis, which is characterized by the high concentration of diterpenics [67, 68, 71]. Its source is most probably a coniferous plant of the genus Cupressaceae (*Cupressus sepmervirens*).. In Portugal both beside poplar propolis is gathered also from gum cistus (C. ladanifer), widely spread in the Meditaranean[27]

In Tunisia, where poplars are not always available, leaf exudates of some *Cistus* spp. act as propolis source[51], while in the Sonoran desert *Ambrosia deltoidea* and *Encelia farinosa* played this role [104]. In Iran, *Ferula* species have been found to contribute to propolis as a secondary plant source, next to *Populus* spp. [100].

Importance of the knowledge of plant sources

The knowledge about plant sources of propolis is not only of academic interest. It could be useful as a basis for the chemical standardization of propolis. Bee glue could be easily characterized using its plant source, which might be established by simple TLC [73].
The knowledge about propolis plant sources is important to beekeepers to be sure that their bees have the proper plants in their flight range. It is known that colonies suffer when they cannot collect propolis, bees are even said to use "propolis substituents" like paints, asphalt and mineral oils which could severely threaten pharmaceutical uses of bee glue [39] .

Propolis from stingless bees

Until now, the investigations on tropical propolis concentrated almost only on *Apis mellifera* bee glue. In tropical South America there are indigenous stingless bee species (*Meliponinae*) Some of them collect resinous material from plants and mix it with beeswax and soil to form the so called *geopropolis* [38]. Others mix the plant resins with was to form the so-called *cerumen*, the building material for their nests (analogous to beeswax in *A. mellifera* hives). A few investigations have been published on this type of propolis which appears to become the subject of an increasing interest[4]. In Venezuelan propolis of stingless bees, prenylated benzophenones predominated, while the composition of geopropolis from three Brazilian stingless bee species was different from this in Venezuela. The main components of these samples were diterpenic acids and triterpenes (alcohols and ketones). A number of flavonoids were isolated in Brazilian stingless bee propolis from two species of Amazona bees[24]. Obviously this is an interesting field for future research activities, especially with respect of the information that stingless bee propolis possesses biological activity similar to that of honey bee propolis.

Poplar propolis

Baccharis or "green propolis"

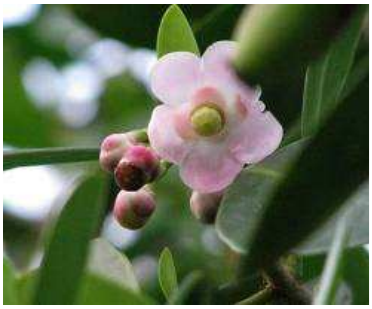
Red Dalbergia propolis

Hermandia nimphaefolia in the Pacific Taiwan, Japan

Dalbergia (Indian rosewood), India, Nepal, Brazi

Platanus acerifolia, Greece

Stem of the mango tree



Flower resin of the Clusia tree in Latin America

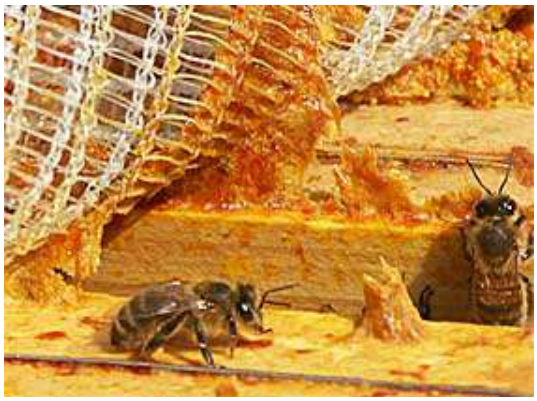
Birch trees in Russia

Besides from poplar and Baccharis, propolis bees gather the glue also in other plants

Table 1: **The most widespread propolis types: plant origin and major constituents**

Propolis type	Geographic origin	Plant source	Main constituents	References
Poplar	Europe, North America, non-tropic regions of Asia, New Zealand	*Populus spp*. of section *Aigeiros*, most often *P. nigra L.*	Flavones, flavanones, cinnamic acids and their esters	3, 9, 29, 35, 69
Green	Brazil	*Baccharis* spp., predominantly *B. dracunculifolia*	Prenylated *p*-coumaric acids, diterpenic acids	83
Birch	Russia	*Betula verrucosa Ehrh.*	Flavones and flavonols (not the same as in Poplar type)	75
Red propolis	Cuba, Brazil, Mexico	*Dalbergia spp.*	Isoflavonoids (isovlavans, pterocarpans)	26, 98
Mediterranean	Sicily, Greece, Crete, Malta,	Cupressaceae (species unidentified)	Diterpenes (mainly acids of labdane type)	67, 68, 71
Clusia	Cuba, Venezuela	*Clusia spp*.	Polyprenylated benzophenones	30, 99
Pacific	Pacific region Okinawa, Taiwan, Indonesia)	*Macaranga tanarius*	C-prenyl-flavanones	18, 33, 41, 42

HARVESTING




Gathering of propolis with a plastic net, placed on the top of the hive
photos courtesy P. Patrice du Sert

In the temperate zones propolis is gathered during summer until the beginning of autumn. Generally, beekeepers collect propolis by scratching off propolis present on the comb frames and in the bee hive box. However, this propolis is not of good quality for medicinal uses. Pure and good quality propolis can be collected with different collection modes.

Bees try always to seal cracks in their hive. This behaviour is used by beekeepers for gathering propolis. For that purpose plastic nets or grids or nets with a mesh diameter of 2 to 4 mm are placed on top of the beehive and the bees seal them.

In temperate climate zones the plastic net is placed on the beehive at the end of the bee season when the bees prepare for overwintering. The net filled out with propolis is taken out and is placed in the freezer. After rolling the net the propolis falls and can be easily harvested. Light, and air circultation stimulate the bees to collect more propolis. That is why, the cover of the hive is opened slightly to allow air circulation and bring light into the hive.

A frequent contact with propolis can cause skin rash. That is why it is safer to use gloves when harvesting and having intensive contact with propolis.

Propolis contains always a certain amount of beeswax, about 20-35 % (see table 2). Beeswax content can be reduced by a method based on differences in specific density and involves the adding of water and microwave heating of a propolis sample[31]

Propolis should be stored closed in dry, dark places. In the frozen state it can be pulverised in mortar to a fine powder.

PROPOLIS STORAGE AND SHELF LIFE

Storage

In general, propolis is fairly stable, but proper storage is important. Propolis and its extracts should be stored in airtight containers in a dark place, away from excessive and direct heat. Over 12 months of proper storage, propolis will lose very little or none of its antibacterial activities. It was shown that propolis ethanol extracts exhibited unchanged antimicrobial activity after 15 years of storage[54]. This stability is probably due to the stability of the phenolic substances, mainly responsible for the antibacterial properties of propolis. Analysis of Brazilian propolis which was frozen for 15 years revealed the same composition, showing that propolis can be stored as frozen without change for a long time[19]

On the other hand, propolis contains aromatic and heterocyclic compounds and flavones and anthraquinones that are sensible to photo-oxdation[97]. Thus propolis should be stored in the dark, ethanol solutions should be made out of brown glass. The freshness of propolis can be determined by measuring the activity of alpha-glucosidase, the activity of which decreases exponentially at room temperatures[105].

Lyophilization (freeze drying) of extracts has been described as a method which preserves the antibacterial characteristics, but nothing has been written about effects of long-term storage of such materials. This method may gain importance for larger scale use and certain formulations, but it is possible that some of the synergistic

characteristics of propolis may be lost during lyophilisation. On the whole, lyophilisation cannot be recommended as a method for propolis preservation as it seems that propolis is quite stable.

The shelf-life of propolis containing products depends very much on their composition and has to be determined for each case. The more some components of a product are susceptible to decomposition, the shorter will be the shelf-life of that product. This is the reason for compromises that are necessary in the selection of artificial and/or natural and traditional ingredients, preservatives and larger production for extended markets. However, propolis and its extracts function as a mild preservative due to their antioxidant and antimicrobial activities and thus may actually prolong the shelf life of some products.

Labelling and shelf life

Approximate figures, estimated from the qualitative data from the literature:

Proteins: max.1 g/100 g
Carbohydrates: max. 1 g/100 g
Fat: max. 1 g/100 g

Shelf life:

- Ethanol extract: three years after packing or the ready product.
- Honey-propolis mixtures: two years after packing of the product.
- Raw propolis can be stored frozen for several years in an air-tight container.

PROPOLIS PREPARATIONS

The application forms shown in this section are adapted after Ludyanski[46]Pochinkova[76], Tichonov et al.[96] and in the online publication of Krell[40], where more recipes can be found.

Tichonov's monograph on propolis preparations

Prof. Tichonov and his team of the Ukranian Kharkov pharmacy faculty, has produced a detailed propolis monograph describing in detail different propolis preparations (poplar propolis) He compared the extraction of phenolic substances in 40, 70 and 95 % ethanol for 24 to 144 hours extraction time (maceration). Best extraction of the phenolics was achieved by 70 % and 95 % ethanol after 144 hours. About 90-95 % of the maximal extraction was achieved already after 72 hours. He also compared also the dependence of the extraction efficacy on the size of the propolis particles, best extraction was found with 0.5 - 1 mm particles. Tichonov developed a fractional-differential extraction method with better efficacy than the traditional maceration method. The extracted propolis is called Phenolic Hydrophobic Preparation (PHP). The aim was to produce a water soluble PHP containing a maximal concentration of the phenolic active ingredients, using detergents. For that purpose he uses Polysobate 20, 40, 60 and 80 (Tween 20) a polysorbate surfactants whose stability and non-toxicity allows it to be used as detergents and emulsifiers in a number of domestic, scientific and pharmacological applications. The oral toxicity dose for the Tween substances is 25 g/kg. A maximal concentration of the phenolic fraction was achieved by mixing 1 part of PHP, 0.3 p Tween 80 and 0.5 p water, a concentration of 55.5 % PHP. Tichonov tested also solubilisation with different non toxic, synthetic non-ionic surfactants. He chose ethylene oxide / propylene oxide copolymers type surfactants with a MW of 5500. Tichonov determined the bioavailabilty and the toxicity of water soluble PHP. Based on his studies different propolis preparations are marketed in the Ukraine[96].

A topical formulation of Brazilian propolis was developed, containing Polowax as a stabilizer against UV damage[49].

Raw whole propolis

The simplest application of is to grind frozen propolis to powder with the help of a mill. Simple coffee mill does the job. The propolis powder can be mixed to honey food or drinks for intake, can be used as a starter for solutions or can be used for the production of propolis pills. Large pieces of propolis can be chewed, but it should be consumed in small quantities. Powder can be made into capsules or mixed with A special form of raw propolis, the so called water soluble whole propolis has been developed by Glenn Perry, www.glennperry.com

Tinctures

Tinctures are prepared in ethanol, glycol and olive oil. The latter two ones are better for skin and cosmetic applications. Ethanol is the best solvent for extracting the bioactive substances (balsam). Propylenglycol can

dissolve less propolis, 20 g per 100 ml glycol can be dissolved[88]. Glycol tinctures are highly antioxidant and can be used in skin protection[48].

Ethanol tinctures

The optimal conditions for propolis extraction have been studied in different publications. In practice propolis is generally macerated (extraction in the solvent without stirring) with occasional shaking. Other extraction methods as Soxlett, ultrasound or microwave or differential extraction are better [22],[96] but need specific equipment and cannot be used under home conditions. 60-80 % aqueous ethanol solutions have a higher biological activity than tinctures, prepared with more or less water[62, 63, 87]. In practice many different propolis maceration procedures are given, the maceration time being sometimes as long as one year. The maceration time for best extraction of bioactive materials depends on different factors: e.g. on the extraction time, extraction method, on the solvent composition, on the propolis concentration and on the size of the propolis particles. Higher temperatures increase the extraction power, but due to the volatility of ethanol room temperature between 20 and 25 ^{o}C are optimal.

Extraction has been studied for poplar propolis in detail by Tikhonov and coworkers[96] who developed a semi-industrial method of differential extraction for optimal and reproducible extraction of propolis. They found that optimum extraction of phenolics is when fine propolis particles (size 0.5-1 mm) are extracted using 95 % ethanol. These particles which already after 3 days optimum extraction of phenolics is achieved under conditions of maceration.

Cunha et al. found out that when using 70 % ethanol the maceration of 20 g/ 100 ml of green propolis is optimal 30 days of extraction time, although there was no statistical difference between the extractions of 10 and 30 days[22]. This has been confirmed in another study on extraction of Baccharis propolis where a minimum of 5 days was suggested[52].

Practical considerations

- *For human use only non-toxic solvents should be used, ethanol of Pharmacopeia quality is the best choice.*
- *The amount of balsam (bio-active, ethanol soluble part of propolis) that can be dissolved is generally not been exactly determined: the percentage of propolis indicates how much raw was originally macerated with propolis. Until 50 % ethanol tinctures are sold on the market.*
- *If the pH of the aqueous ethanol is fixed at 8.0 more flavonoids can be extracted[52]*
- *Propolis should be pure, remove coarse debris and excessive wax.*
- *Place propolis in freezer and break it in small pieces or mill it to powder for a better solubility*
- *60-80 % aqueous ethanol solutions have a higher biological activity than tinctures, prepared with more or less water[62, 63, 87] 70 % Propolis is most widely used,*
- *Store vessel in the dark for about two weeks, shaking occasionally, more than 2 weeks brings only a small improvement of yield.*
- *Filter through a paper filter (coffee filter will do) and store tincture closed in a clean dark vessel. If vessel is not brown or reddish, store in the dark, or pack vessel in aluminium foil.*
- *Ethanol-free propolis can be made by evaporating the ethanol in a water bath. The remaining pure balsam can be mixed to honey or other materials where ethanol-free material is required.*

Propolis ethanol tincture:

- *Add 100 g propolis to 400 g 70 % ethanol (for 20 % tincture)*
- *Store vessel in the dark for at least two days, better one or two weeks, shaking occasionally (the longer the extraction time, the greater the concentration of active ingredients, but more than 2 weeks does not bring more benefit)*
- *Filter through a paper filter (coffee filter will do) and store tincture closed in a clean dark vessel. If vessel is not brown or reddish, store in the dark, or pack vessel in aluminium foil.*

Glycol and olive oil tinctures

Glycol

Propolis powder can be macerated with propylene glycol, the maximum being 10 g/100 ml, for 2-4 weeks, and then filtered[79, 86]. Higher propolis concentration can be achieved by evaporation of ethanol tinctures and

replacing ethanol by glycol. 30 % propolis solution was used in wound and burn treatments[12]. This solution is better for use as mouth and nose sprays. Note that this solution is only for **external use.**

Olive oil

Olive oil alone does not extract enough propolis. Only about 5 g propolis per 100 ml can be dissolved. The more propolis can be dissolved by adding 96 % ethanol: e.g. propolis 30 g, olive oil 70 ml, ethanol 96% 60 mL [78]

Propolis water extracts

Water soluble propolis

A patent described by Sosnowski[93], based on the extraction of poplar propolis:

Propolis is prepared and extracted as described in method 1 but using a 10-25 % ethanol solution, though many other solvents are mentioned in the patent application. After 1 to 10 days at 0 to 37 C (preferably towards the warmer temperature limit) with periodic agitation, the solution is filtered for the first time through Whatman No. 1 filter paper, or a double layer of very fine cotton cloth. The filtrate is cooled as much as possible (without freezing) for 24 hours and is then filtered again, cold, through a Whatman No.50 filter paper. A third and final filtration may be carried out cold or at room temperature with a 2 µm filter. Finally, the solvent is removed by evaporation or freeze drying.

Water extract after Ludyanski[46]

- *300 ml of water is poured in a pan over 30 g propolis, cut in small pieces.*
- *Close pan and boil gently for 40-45 min.*
- *Cool down, collect wax from the surface and decant supernatant (1).*
- *Add a new portion of 30 g propolis pieces to remaining precipitate in the pan and 300 ml of cold water.*
- *Boil gently for 10-15 minutes*
- *Cool down, collect wax from the surface and decant supernatant into vessel with supernatant 1 to give about 500-600 ml of propolis water extract*

Simple extraction with water

- *Add 50 g of propolis to 100 ml of water*
- *Boil for 60 minutes*
- *Cool down to room temperature and filter*

According to Ludyanski this water extract has an antifungal, antibacterial effect and also other known biological effects. This water is ready for drinking. Keep in a dark place.

For extraction methods like this one and others, where the final product is a paste or powder, the initial proportions of propolis and solvent are not very important. Much larger quantities of propolis can be used for quicker extraction, e.g. 500 g propolis in 1000 ml solvent. However, sufficient active ingredients usually remain in the filter residues to justify another, longer extraction with clean alcohol. A few recipes using the dried powder are mentioned at the end of this chapter. No scientific publications or studies were cited by Sosnowski (1984) concerning the efficacy or biological activity of this extract, though he claims that the antioxidant properties of the propolis extract from concentrated ethanol or diluted ethanol are the same.

Propolis can be macerated with water by stirring 2.5 g/100 ml

Extraction at 30°C with water with a subsequent nanofilter filtration lead to a good extraction of Baccharis propolis[53] A minimum of 5 days and a adjustment at pH 8.0 has been suggested for optimum water extraction of Baccharis propolis [52] but the extensive studies with aqueous ethanol suggest 30 days for optimum extraction of the same propolis type[22]

Propolis pills and semisolid preparations

Grind finely deeply frozen pure whole propolis with a cold mill. Mix propolis powder with lactose, e.g. 1: 1 and press into pills.

A Lithuanian semisolid preparation with a soft propolis extract (patented) with olive oil and cocoa butter is described[80]

Krell[40] describes a preparation of a propolis paste based on the evaporation of an ethanol extract. This paste can be used itself or added to different types of butter.

Mixtures, emulsions, concentrates, creams, ointments

Ethanol-water mixtures

- *Mix 1 part 30 % propolis ethanol tincture with 5, 10 or 100 parts of water.*

Some the propolis constituents will precipitate. The durability of this mixture durability is limited to 7 days. Store in the dark. Shake before use. Used for stomatology and for compress.

Ethanol-oil emulsion:

- *Mix 1 part 30 % prop ethanol tincture with 1 or 2 parts of glycerol or edible oil.*
  Store in the dark. This emulsion has an indefinite shelf life.

Propolis concentrate

There are propolis concentrates with 25 % liquid ("wet" concentrate) and 5 % liquid ("dry" concentrate. The concentrates are prepared by the ethanol of a 30 % propolis ethanol tincture 1 at 60 ^{o}C in water bath (see above). These concentrates are used for the preparations of creams, pastes and supositoria, or for mixing it to honey.

Propolis creams and ointments for different uses

Creams on the basis of a propolis concentrate

- *Use vaseline or vaselin-sunflower (2:1) oil and lanoline as an emulgator. In practice 1,2 and 5 % propolis are used.*
- *1 and 2 % cream: add 90 g vaseline, 10 g lanoline to 1 or 2 g of a dry propolis concentrate.*
- *5 % cream: add 80 g vaseline, 15 g lanoline to 5 g of a dry propolis concentrate.*
- *While mixing lanoline with a spatula add first propolis concentrate unitil a uniform mass is attained, then add vaseline and mix well.*

For the basis of the cream vaseline and lanoline in proportions 9:1 and 8:2 are used. For 100 g of this basis 10-20 ml of 30 % propolis ethanol extract are used:

- *warm up basis in a water bath (at about 40-50 ^{o}C) and add propolis extract*
- *while stirring, warm to boiling to evaporate ethanol*
- *While still warm sieve cream, containing 3 or 6 % propolis and pack it in a dark cream box, tightly closed.*

Propolis paste

Place propolis in freezer, cut it into small pieces and ground it to a fine powder. Mix it in a vessel with the basis (honey, margarine, butter etc.), so that 5, 10, 15 and 20 % propolis cream is obtained. Also, the dry concentrate can be used (5 g dry propolis concentrate for 100 g basis). The dose to be taken is 3 times a day, take a tea spoonful 0.5-1 hour before meals.

Propolis butter

- *Boil 1kg of butter and cool down to 80 ^{o}C*
- *Add 150 g propolis powder and mix well*
- *Cover with lid and wait 20 min. while stirring from time to time, in order to prevent propolis from stirring to pan.*
- *Extract propolis into butter by heating mixture at 80-90 ^{o}C while energetically stirring*
- *Filter hot mixture through a gaze and keep closed in a cool dark place until consummation.*

The dose to be taken is 3 times a day, take a tea spoonful 0.5-1 hour before meals.

Propolis cream for dentistry, ingredients (in parts by weight) after[93]

10	*Lanolin*
10	*Unbleached beeswax*
10	*Petrolatum (or Vaseline, the trade name for a petrolatum)*
2	*Ethyl aminobenzoate*
3	*Clove oil*
15	*Propolis (50% EEP)*

COMPOSITION

The present standard methods for propolis analysis have been described in a review publication by Bankova et al. in 2016[2].
Propolis is composed mainly by the plant resins and exudates that bees gather. Bees add wax, and also some secretions and pollen to it. The composition of propolis depends on its botanical and thus also on its geographical origin.

Several hundred different compounds have been characterised in the different propolis types. The typical components of poplar propolis are the phenolics: flavonoid aglycones, (flavones and flavanones), phenolic acids and their esters, as the volatiles. The typical compounds of Brazilian propolis are prenylated derivatives of p-coumaric acid and of acetophenone, as well as diterpenes and lignans. The flavonoids are different from those found in 'poplar type' propolis.

The overall content of this propolis type is similar to the poplar propolis, basically containing balsamic and non balsamic components. It contains a main part of plant derived substances and minor part of bee and pollen derived substances. The chemical composition is, however very different.

The balsam part of poplar propolis originates from the collected glue, while the non-balsamic constituents are added by the bees (wax, minerals, carbohydrates etc.)

The non-ethanol soluble part of the Baccharis propolis originating partly from the plant, besides a part of minor constituents originating partly from the plants and from the bees and from pollen.

Table 2 A**: Composition of raw poplar propolis** after [7, 8, 10, 23, 40, 66, 77, 96]

	Substances
BALSAM 40 - 70 % Ethanol soluble Poplar origin	*Phenolics* Phenols, phenolic acids, esters, flavanons, dihydroflavanons, flavons, flavonols, chalkones, phenolic glycerides ; *Others*: Aliphatics: acids, alcohols, esters, aldehydes, ketones, benzoic acid and esters
Essential oils 1-3 % ethanol soluble	Mono-, and sesquiterpenes, aromatic compounds
NON-BALSAM Ethanol insoluble Wax**:** 20-35 % Beeswax origin	Beeswax components
Others**:** ca. 5 % partly ethanol soluble bee and pollen origin	Mainly minerals average ash content 2.1 % Polysaccharides:2 % Proteins, amino acids, amines and amides: 0. 7 % Traces of carbohydrates, lactones, quinones, steroids, vitamins

Table 2 B: **Composition of raw Baccharis propolis** after [7, 17, 22, 47, 64, 65, 85]

	Substances
BALSAM 45 - 70 % Ethanol soluble Baccharis origin	Mainly cinnamic acid and derivatives, coumaric acid, prenylated compounds, artepillin C Minor quantities of phenolics as flavonoids, benzoic acid, aliphatic acids and esters
Essential oils 1-3 %	Sesquiterpenes, aromatic compounds
NON BALSAM	
10-15 % Ethanol insoluble Baccharis origin	prenylated compounds, alkanes and terpenoids
15-25 % Ethanol insoluble Beeswax origin	Beeswax
ca. 5 % partly ethanol soluble Bee and pollen origin	2.5 – 4.5 % minerals 1-2 % of carbohydrates: fructose, mannose, inositol, erythrose 1-2 %: glycerol, lower aliphatic acids, amino acids, amines

QUALITY TESTING AND STANDARD

Propolils has a different status in different countries. In some European countries (Germany, Switzerland) it is considered a medicine while in many others (Austria, France, Spain, USA, Japan, Brazil) it is considered a food supplement. There are good arguments that, similarly to another beehive product royal jelly, it can be considered as food supplement (see report on Propolis and Health). Here the requirements of the food law and the respective quality testing will be considered.
Poplar propolis can be tested by first evaluating its sensory and physico-chemical properties. Poplar propolis can be tested according to the scheme given in the table below.

Sensory, physical und chemical properties of poplar propolis

Sensory properties

- *consistence*: at temperatures higher than 30° C propolis is soft and sticky., below 15° C it is hard and brittle.
- *odour*: pleasant, resinous
- *taste*: bitter, acrid.
- *colour*: varies depending on the botanical origin: brown-yellow, brown -green or brown –red to dark-red

Physico-chemical properties

- *density*: 1.11-1.14.
- *melting point:* 80-105 °C
- *solubility*: slightly soluble in water; low molecular components soluble in ethanol, waxes are insoluble; solubility increases in mixtures of different solvents, e.g. ethanol-chloroform, ethanol-toluene

Propolis authenticity, standard, quality

In quality testing the first important issue is to check the origin of the propolis (authenticity of origin) GC-MS is the method of choice to determine the botanical origin of propolis[34]

Some attempts have been made for the establishment of quality criteria of propolis. The purity and the wax content of propolis have been used as quality parameters of most researchers and these should remain a part of a future propolis standard. In the Bulgarian standard and also some other Eastern European standards (1983) the saponification-, ester and iodisation numbers are used [89]. However, these quality parameters relate only to the wax and resin content in propolis. These components play only a minor role in the biological activity of propolis.

As mentioned above, the composition of propolis differs greatly depending on its botanical origin. The measured compounds should be important from biological points of view. Standards for poplar and green propolis have been proposed. The poplar propolis is based on the quantification of the main biologically active substances, balsam (the ethanol extractable fraction), phenolics and flavonoids.
Poplar propolis can be adulterated with poplar bud glue, this can proven by measuring catechol and catechol oxidase[32]

Table 3**: Standard proposal for poplar propolis**[70] and the **Brazilian standard for green propolis** [47].

Component	**Poplar Propolis min Values, g/100 g**	**Green Propolis Min Values g/100 g**
1. Balsam	45	35
2. Total phenolics	21	7
3. Total flavones and flavonols	5	-
4. Total flavanones and dihydroflavonols	4	-
5. Total flavonoids (3+4)	9*	1
6. Beeswax	Max. 25	Max 25
Insoluble matter	Max. 5	Max. 5
Ash content	Non specified	Max 5

Good propolis quality means

- low content of mechanical matter (wood, dead bees, etc.)
- no or minimal contamination by pesticides and heavy metals (best quality organic one)
- high balsam content
- high content of biologically active compounds (according to the chemical type)
- low wax content

Other propolis types have different sensory properties. Birch and Brazilian Baccharis propolis have greenish colour, while the red propolis from the tropics is redish.
The determination of the propolis origin is the first necessary step, the composition of propolis being typical for each propolis type.. Recently a simple TLC method was developed for a quick estimation of propolis origin, which can be used as a screening method[13] The quantitative composition is determined mostly by chromatographic analysis (GC or HPLC with subsequent MS detection) [5, 6, 34]

Propolis quality and harvesting method

In 2010 Italian researchers characterised chemically propolis harvested with different methods: by scraping, with plastic mats or with wooden wedges. The propolis harvested with wooden wedges had the highest content of balsam and the highest concentration of phenolics, thus the best quality[60, 61]. An Argentinian research paper recommends plastic matts as the better harvesting method than scraping, as it has lower Pb contamination[84], while a Columbian one says that the physico-chemical and biological characteristics are better in the plastic matt collected propolis than in the scraped one[50]

In France it is recommended that collection is from Spring to Autumn. Propolis that has been in the bee hive during winter is much darker and is of lesser quality (Percie du Sert, personal communication).

Contaminants

This topic is reviewed by Bogdanov. Most important contaminants are heavy metals and lipophylic synthetic acaricides used for varroa mite control. [14].

Minimal contamination can be guaranteed by using certified organic propolis.

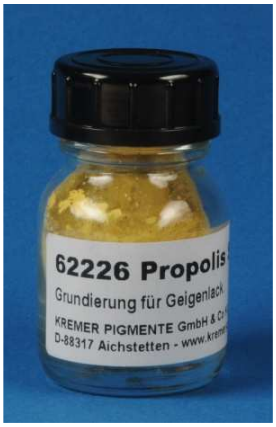
USES AND TRADE

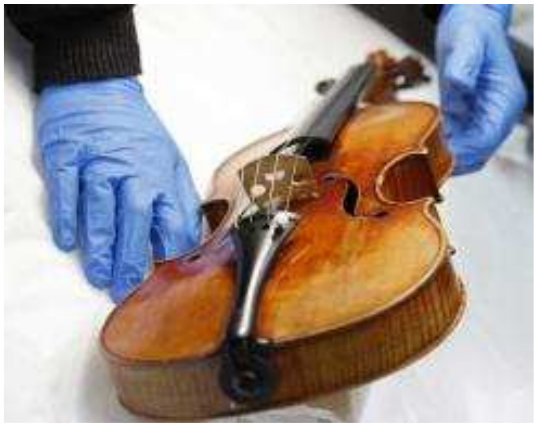
Uses

Propolis is mostly used in natural medicine as a health enhancing food supplement, in medicine, dental and veterinary medicine, in cosmetics, as food preservative or antioxidant or as a phytoinhibitor in agriculture (see Chapter 2). It has been also used as air disinfectant[59].

Propolis posseses interesting physical properties that make it a suitable component of wood varnishes [16]. Propolis probably has been more commonly used in wood preservatives or varnishes and it is claimed that the famous violins of Stradivarius contained propolis[36]. The propolis of the stingless bees called geopropolis was used by Amazon Indians as arrow cement[94].

Because of its acaricide and antibiotic activity propolis been proposed to be used against varroa[25] or American foul brood[1]





The famous violins of Stradivarius contained propolis, now propolis is an ingredient of violin garnishes.

Trade

There is no official information on crude propolis trade.

According to Crane, p.551 of [20] in 1984 following export figures are reported:

China: 35 tons, Romania 19 tons, Argentina, Chile and Uruguay: 7, 8 tons; Canada: 3-4 tons.

Brazil produced in 2004 250 tons per year[58], while in 2009 propolis production in China was 300 tons per year[45].

After a personal communication of Hartmuth Schreiter following unofficial and unconfirmed figures are given for other producers: Argentina and Uruguay: 5-10 tons; Baltic states: ca. 2 tons; Poland: ca. 2 tons.

Presently the world propolis price varies according to origin and quality: Chinese propolis is offered at prices of about 25-50 euros per kg, while Brazilian propolis has higher prices, between 100 and 150 euros per kg.

Customers should be cautious when buying propolis from the Internet. It is better to buy propolis from companies with experience with trading with propolis, e.g. www.allwex.de , www.beevitalpropolis.com

It is important to demand analysis of quality criteria, including compliency to residue standards. Organic propolis is a natural choice for a product with a minimal contamination and optimal biological activity.

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