Phytochemistry 155 (2018) 1–11

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Review

The phytochemistry of the honeybee

Vassya Bankova*, Milena Popova, Boryana Trusheva

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 9, 1113 Sofia, Bulgaria

ARTICLE INFO

Keywords:
Honeybee
Propolis
Propolis plant sources
Metabolomics

ABSTRACT

Honeybees rely on plants for everything they need to keep the colony running; plant nectar and pollen are their only carbohydrate and protein food sources. By foraging to satisfy their basic nutritional demand, honeybees inevitably gather specialized plant metabolites as part of the nectar and pollen. In general, these compounds possess biological activity which may become relevant in fighting pests and pathogens in the hive. The third plant derived bee product, besides honey and bee pollen, is propolis (bee glue), which comes from plant resins. It is not a food; it is used as a building material and a defensive substance. Thus, the beehive is rich in specialized plant metabolites, produced by many different plant species and the expression "Phytochemistry of honeybees" is not inappropriate. However, it is virtually impossible to perform a detailed overview of the phytochemical features of honey and pollen in a review article of this nature, for reasons of space. The present review deals with propolis, because it is the bee product with highest concentration of specialized plant metabolites and has valuable pharmacological activities. The most recent developments concerning plant sources of propolis, bees' preferences to particular plants, the application of metabolomic approaches and chemometrics to propolis research and the problems concerning standardization of propolis are summarized. The overview covers the literature published in the last decade, after 2007.

1. Introduction

Honeybees *Apis mellifera* L. have been living on Earth for over 125 million years, probably for as long as flowering plants. Nowadays bees are at home in almost all habitats in the world: from the equatorial rainforests and tropical deserts to the Subarctic regions of Eurasia and North America. This remarkable evolutionary success is as much due to the sophisticated social organization of honeybees as it is due to the products which honeybees manufacture, products which are essential for the life of the colony, serving as food, building materials and defensive weapons. Three of these products: beeswax, venom, and royal jelly, are chemically synthesized by the bees themselves. The other three - honey, pollen and propolis - are derived from plants and are modified by the bees for their own use.

Actually, honeybees rely on plants for everything they need to keep the colony running. The long co-evolution of bees and flowering plants has resulted in a complicated and mutually beneficial bee-plant relationship, combining activities of two very different types of genetic inheritance. For the honeybees, plant nectar and pollen are the main carbohydrate and protein food sources (Erler and Moritz, 2016). On the other hand, plants need pollinators and thus they have to attract bees, and in order to do so they offer different kinds of rewards. Floral nectar represents the main plant reward for many pollinators, including honeybees. Nectar is basically a sugar solution; the three most common nectar sugars are the disaccharide sucrose and the two hexoses glucose and fructose (of course, there are some differences between different plant species) (Chalcoff et al., 2005). Bees make honey mainly from the nectar by evaporating water from it, and while doing so secrete into it enzymes: invertase and glucose oxidase. The sucrose in the nectar is inverted to glucose and fructose in the bee's stomach through the action of the enzyme invertase. Preservation of honey is achieved through evaporation of water and hydrogen peroxide formation by the action of glucose oxidase on glucose. Honey is the bees' carbohydrate food source, while the most important source of proteins for bee survival is bee pollen. Collected by worker bees flower pollen is accumulated as pellets (corbicular pollen) in pouches on the rear legs of the bee and it is the mixture of these pellets that comprises bee pollen, which supplies the colony with proteins and amino acids (De-Melo and de Almeida-Muradian, 2017). Pollen also contains fat, vitamins, microelements, etc.

However, plants need to provide pathogen-free food for the bees if co-evolution is going to be a success (Erler and Moritz, 2016). Hence, the nectar offered by a flower should not be fermented nor should pollen be contaminated with fungal pathogens. It is therefore not surprising to see plants adding antibiotic specialized metabolites to the

* Corresponding author.
E-mail addresses: bankova@orgchm.bas.bg (V. Bankova), popova@orgchm.bas.bg (M. Popova), bobi_tru@orgchm.bas.bg (B. Trusheva).

https://doi.org/10.1016/j.phytochem.2018.07.007
Received 28 March 2018; Received in revised form 9 July 2018; Accepted 16 July 2018
0031-9422/ © 2018 Elsevier Ltd. All rights reserved.

nectar to prevent bacterial fermentation (Stevenson et al., 2017), and that pollen also contains antimicrobial specialized metabolites, mainly phenolics (Ares et al., 2018). It is well known that bee pollen shows characteristic flavonoid patterns according to the plant origin. While foraging to satisfy their basic nutritional demand, honeybees inevitably gather specialized plant metabolites as part of the nectar and pollen. In general, these compounds possess biological activity which may be relevant for fighting pests and pathogens in the hive.

Unlike honey and pollen, the third plant derived bee product, propolis (bee glue), is not a food. Bees use it as both building material and defensive substance. They make use of the mechanical properties of this resinous material by applying it for blocking holes and cracks, repairing combs, strengthening the thin borders of the comb, etc. On the other hand, they make use also of its biological action: bee glue contains the putrefaction of "embalmed" intruders, killed in the hive and too large to be carried out, and it is responsible for the lower incidence of bacteria and moulds within the hive than in the atmosphere outside (Simone et al., 2009). To produce propolis, bees collect vegetable material and mix it with wax. It is now generally accepted that bees collect 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, mucilages, gums, resins, latices, etc. (Crane, 1990). In some cases, bees may also cut fragments of vegetative tissues to release the resin used in propolis production (Salatino et al., 2005). Numerous plant species secrete highly antimicrobial resins to protect vegetative apices, young leaves and wounded tissues, and such secretions provide propolis raw material for bees. But why do bees need a special antimicrobial material? Honeybees battle an extensive assemblage of pathogens using both individual and "social" defenses. The collection of resins - complex plant secretions with diverse antimicrobial properties, acts as a colony-level immune defense for honey bees. Foraging for resins is energy demanding and provides no clear direct reward for the individual foraging bee, so resin collection and use functions as an aspect of the so-called "social immunity" (Simone et al., 2009).

The beehive is rich in specialized plant metabolites, produced by many different plant species, so the expression "Phytochemistry of honeybees" is not inappropriate. However, it is virtually impossible to perform a detailed overview of the phytochemical features of honey and pollen in a review article of this nature, for reasons of space. In addition, specialized metabolites are minor components of pollen and honey. The present review deals with propolis, because it is the bee product containing the largest amount of specialized plant metabolites (at least 50% of its weight). Moreover, it is not surprising that propolis, originating from protective plant secretions and serving as a defensive substance in the beehive, is also active against human pathogens. This has been proved by numerous publications, reporting the results of well-designed and reliable experiments (Burdock, 1998; Seidel et al., 2008). It has been demonstrated that propolis also possesses many other beneficial pharmacological actions (recent reviews: Sforcin, 2016; Pasupuleti et al., 2017; Zabaiou et al., 2017).

2. Propolis production by honey bees

Only a small number of worker bees collect resin for propolis production. According to Simone-Finstrom and Spivak (2012) "overall resin foragers consistently represent a minority of the foraging force (1% or less of total number of foragers)". The process has been described in detail in the works of Meyer (1956) and Morse (1975). They found out that only a few of the workers, not older than 15 days old, are specialized in propolis foraging. The resin forager bee cuts off a particle of resin (or resin containing plant tissue) with the mandibles, then transfers it to the corbicula on the hind legs (where bees carry pollen loads). After that the resin is brought back to the nest where it is mixed with varying amounts of wax and used as propolis. Foraging for resins is typically observed in the afternoon on sunny days, likely due to the increased pliability of resins at higher temperatures. The resin collection activity is most frequent in late summer through autumn when the honey flow is significantly reduced (Alfonsus, 1933; Meyer, 1956; Crane, 1990).

Bees have their ways of finding plant resins rich in biologically active phytochemicals (Salatino et al., 2011). The secretion of antimicrobial material is widespread in the plant kingdom; various plant species secrete highly antimicrobial resins to protect vegetative apices and young leaves, wounded tissues, etc. (Shuaib et al., 2013). However, there is a strong asymmetry between the wide available diversity of plants producing resin with high bioactivity, and the narrow spectrum of plant sources of propolis established in all parts of the world. Indeed, in a particular geographic region, bees usually demonstrate obvious preference for one or two source plants (Bankova et al., 2006). The cues used by resin foragers for finding a resin source are virtually unknown, although it is clear that they select specific sources. One leading hypothesis is that volatile compounds released from the resin play an important role in bees locating resin sources, signaling the availability of a material rich in bioactive compounds (Simone-Finstrom and Spivak, 2010). It is well known that worker honeybees respond to odors (chemical signals) in several behavioral contexts (Winston, 1987; Salatino et al., 2005). Workers learn the association of floral odors with the nectar and/or pollen rewards offered by the flowers, thus allowing for future identification of the plants from which food can be harvested (Smith and Cobey, 1994). So far no data has been gathered about the role of odors in foraging for propolis. It is possible that the same learning process occurs in the case of resin collection by honeybees (Bankova et al., 2014), taking into consideration the finding of Leonhardt et al. (2010), that stingless bees in Borneo use volatile terpenes as olfactory cues to find appropriate resin sources.

According to another recent hypothesis, formulated by Salatino and Salatino (2017) there seems to be a significant "constraint that honey bees have to face, a barrier that probably limits the number of plant species providing propolis resin". Some materials with good antimicrobial properties, such as latices, resins, and gums, are too sticky and hard to be collected by resin foragers. Bees normally gather exudates which form smooth solid films, which can be scraped by the bees from the plant surface and attached to their hind legs. Most of the materials containing antimicrobial specialized metabolites are too hard to be cut with the mandibles of honeybees, because their mandibles are delicate, as compared with mouth parts of typical chewing insects. A finding that probably confirms the importance of the mechanical properties of the source resins for propolis production is in the article of Simone-Finstrom et al. (2010), who proved that resin foragers were better able to learn tactile stimuli and discriminate between a rough and a smooth surface as compared to pollen foragers. The plant materials conventionally called "resins" in propolis research, are sufficiently soft and smooth to be either cut (apical buds and leaf primordia) or scraped from the plant surface (exudates) by the honeybees with their delicate mandibles (Salatino and Salatino, 2017).

3. Propolis plant sources

The identification of propolis plant source(s) is important, since propolis is one of the commercial bee products and contributes to the income of beekeepers. The knowledge of the plant sources can help to increase propolis production and to attain higher degree of standardization. Also, if appropriate sources are insufficient in the vicinity of the hive, bees have been observed to collect resinous matter from asphalt, fresh paint, etc., and such propolis is polluted (Alqarni et al., 2015).

Knowledge of the vegetable sources is also essential with respect to the health of the bee colony: in the last decade propolis collection and application by honeybees was identified as an important element of the "social immunity" of the colony. The term "social immunity" summarizes all antiparasitic and anti-infectious colony-level mechanisms

resulting from social behaviour. Propolis was found to be beneficial for the honeybees' immune system, engendering colony level therapeutic effects against some bee pathogens, as a detoxifying agent or primer of detoxification pathways, as well as to increase bee longevity via antioxidant-related pathways. The availability of propolis sources is therefore essential for the wellbeing of the colony (Simone-Finstrom et al., 2017).

Identification of the source plants is not an easy task. In 1990, Crane listed over 60 plant species, reported as propolis sources by different authors based on observation of bees "collecting sticky material on leaf buds of certain trees" (Crane, 1990). This approach however proved to be only partly successful. Later studies based on modern technologies could only confirm 14 of these species as actual plant sources.

In the last decades of the 20th Century, chemical analyses of propolis became more detailed and the chemical comparison of plant resins and propolis provided solid evidence for the particular sources in different regions. In this way, the importance of silver birch *Betula pendula* Roth, trembling aspen *Populus tremula* L. and black poplar *Populus nigra* L. as propolis-bearing plants in Europe was stated and confirmed (Popravko and Sokolov, 1980; Greenaway et al., 1990; Bankova et al., 2000; Isidorov et al., 2014). Later on, the combination of observation and filming of bee behaviour and direct comparison of chemical profiles of both plant material and propolis, in some cases completed by chemical profiling of the resinous material on the bees' hind legs (corbiculae) provided the ultimate proof of the source plant(s). This approach was first developed by Kumazawa (Kumazawa et al., 2003; Shimomura et al., 2012) and further used by the group of Salatino in Brazil (Teixeira et al., 2005) and by some Australian researchers (Tran et al., 2012; Aminimoghadamfarouj and Nematollahi, 2017) This methodology resulted in the unambiguous identification of the propolis sources in Okinawa (*Macaranga tanarius* (L.) Müll.Arg.), Jeju Island, Korea (*Angelica keiskei* Ito) and Kangaroo Island, Australia (*Lepidosperma* spp., *Acacia paradoxa* D.C. and *Myoporum insulare* R. Br.).

Some authors have been trying to use palynological analysis for identification of the plant sources of propolis, as is done for honey (Temizer et al., 2017; Castro-Falcón et al., 2016; Çelemli et al., 2016; Vargas-Sánchez et al., 2016; Silva et al., 2013; Dias et al., 2012). This approach, however, is "dangerously misleading" (Salatino et al., 2005) because resins are usually deposited on buds, young leaves, bark, etc., and not in the vicinity of the anther where pollen can be found. It is impossible to know with certainty whether a detected pollen is the result of a visit aimed at collecting resin or just a contamination from material obtained by bees for other purposes. Pollen grains adhere to the resin during its collection by the bees or from harvested pollen inside the beehives (Barth and da Luz, 2009). A very good illustration of this danger is the recent work of Guzelmeric et al. (2018) who stated that propolis of unambiguously poplar origin (direct chemical comparison) does not contain significant amounts of poplar pollen. On the other hand, it is very much possible to characterize the environmental conditions and the vegetation around the apiary using pollen analysis. Pollen content reflects the flora of the region of origin, and thus palynological analysis can be used to identify the geographical origin of propolis (De Freitas et al., 2011; Matos and dos Santos, 2017).

There is another important question connected to the origin of propolis and it concerns the chemical contribution of the honeybees. Of course, the wax in propolis is mainly, or almost entirely, beeswax (Negri et al., 2000), although many plant resins/exudates contain wax and the plant wax contribution should not be totally ignored. But what else do bees add to propolis? As bees take the plant resin with their mandibles, inevitably some salivary enzymes are mixed with it. Indeed, a few publications report the presence of small amounts of enzymes in bee glue: α-amylase, β-amylase, maltase, some esterases (Kaczmarek and Dębowski, 1983; Kędzia, 2011). β-Glucosidase activity in propolis was also reported however, the authors did not identify the source of this enzyme and speculated that it might have also come from the plant source, and not necessarily from the bees (Zhang et al., 2011a). Much speculation can be found in the literature about the "partial digestion" of plant material by bees' enzymes during the production of propolis. However, there is no positive evidence of such chemical changes. On the contrary, several experiments based on parallel analysis of plant resin taken from the source plant, resin taken from bees' corbiculae (collected resin) and propolis from the beehive ("processed" resin with added wax) have demonstrated that their chemical profiles are identical and no chemical changes can be detected in the specialized plant metabolites of the resin (Teixeira et al., 2005; Tran et al., 2012). Bolstering this argument is the fact that enzymes are unlikely to have any activity in resins/exudates (and propolis) due to the low water content and very high phenolic content.

There are a couple of articles which describe distinct chemical differences between poplar type propolis and poplar bud extracts, claiming that these differences indicate the interference of bee's enzymes. Both articles were intended to identify chemical markers for detecting Chinese poplar propolis adulteration with poplar tree gum. Indeed, the authors have demonstrated chemical differences between poplar buds extract (poplar gum) and poplar propolis: propolis contained no salicin (Zhang et al., 2011b) and no catechol (Huang et al., 2014), whereas these two compounds were present in extracts of poplar buds. However, the extracts of buds containing salicin and catechol were obtained from the ground buds, i.e. from the plant tissue, not from the resin on their surface. This cannot be accepted as positive proof that the resin (exudates), the actual material collected by bees, contains catechol and salicin. As a matter of fact, the protocol for extraction of poplar bud exudates has been described by Wollenweber back in the 1980's: brief extraction of the buds with acetone in order to dissolve the lipophilic exudate material, and no substances from inside the bud tissues (Wollenweber et al., 1987).

As already mentioned, the buds of black poplar, trembling aspen and silver birch were the first chemically proven "suppliers" of propolis in France and Russia back in the 1970's (Lavie, 1976; Popravko, 1978). Through the years, it became clear that these trees are the source of propolis over large parts of Europe, including Great Britain, Switzerland, parts of Italy, Hungary, Czech Republic, Romania, Bulgaria, Macedonia, Poland, Scandinavian countries, Baltic countries (Bankova et al., 2000; Isidorov et al., 2016). Isidorov et al.'s (2016) study revealed that even if resin sources other than black poplar, trembling aspen and birch (such as horse chestnut, black alder or Scotch pine) are readily available, bees do not collect resin from them. Yet in regions where the black poplar is not native but introduced, like Argentina (Isla et al., 2005), South Africa (Suleman et al., 2015) and New Zealand (Markham et al., 1996), honeybees use poplar bud resin for propolis production. In addition, it has been demonstrated that other *Populus* species play the same role in Asia and North America. In Table 1, the new data (published after 2006) about *Populus* species identified by chemical comparison as propolis sources, and new locations where poplars were identified as a propolis source, are summarized.

However, honeybees are widespread also in sub-tropical and tropical regions where no *Populus* species grow. The biodiversity in these phytogeographic zones gives bees a great choice to find appropriate resin sources. The best known tropical propolis-bearing plant is the source of Brazilian green propolis, *Baccharis dracunculifolia* DC (Salatino et al., 2005). In the last decade, the chemical analysis of propolis from many other locations outside the Temperate Zone lead to the discovery of new chemical types of propolis, and respectively their sources have been identified as new propolis source plants. A list of propolis sources, identified by chemical evidence, is presented in Table 2.

It is obvious that bees choose appropriate resin sources from different plant families, according to their availability at the specific location and their suitability to the needs of the bees. It seems that the most preferred family is *Fabaceae*, with at least five different species proven to be sources of bee glue. Another interesting observation is the fact that bees collect *Mangifera indica* resin in a wide range of

Table 1
New data (after 2006) about *Populus* species as propolis sources, and new locations where poplars were identified as propolis source.

Populus species	Country	Chemical markers	References
P. nigra L.	Germany	Flavonoid aglycones, phenolic acids and their esters	Bertrams et al., 2013
	Turkey		Guzelmeric et al., 2018
	Serbia		Anđelković et al., 2017
	Crimea		Isidorov et al. 2014
	Belarus		Isidorov et al. 2014
	Algeria		Piccinelli et al., 2013
	Argentina		Kumazawa et al., 2010
P. tremula L.	Germany	Phenolic acid glycerols	Bertrams et al., 2013
	Serbia (over 500 m altitude)		Anđelković et al., 2017
P. tremuloides Michx.	Canada, Richmond	*P*-coumaric and cinnamic acid	Christov et al., 2006
P. canadensis Moench (hybrid variety of *P. nigra*)	China	Caffeic acid esters, pinobanksin and its derivatives	Hu et al., 2014; Wang et al., 2018
P. balsamifera L.	USA, Oregon and California	Dihydrochalcones	Aliboni, 2014
	USA, Minnesota	Fingerprint comparison: GC-MS	Wilson et al., 2013
P. deltoides W.Bartram ex Marshall	Brazil, the mid-South of State of Paraná	Fingerprint comparison: GC-MS	Adelmann et al., 2007
	USA, Minnesota	Fingerprint comparison: GC-MS	Wilson et al., 2013
P. trichocarpa Torr. et Gray	Canada, Victoria	p-hydroxy-acetophenone, benzyl hydroxybenzoate	Christov et al., 2006
Populus spp.	Mexico	Pinocembrin, pinobanksin 3-*O*-acetate, galangin, chrysin	Li et al., 2010

geographic locations in Africa, South-East Asia, the Middle East, and South America (Table 2.).

In recent years, the number of propolis plant sources, positively identified by chemical analysis, has grown rapidly. Yet there is a lot of work to be done in order to reveal the sources of many propolis types with specific chemical composition, different from all the known source plant resins. There are many of them, for example Portuguese propolis rich in flavonoid glycosides (Falcão et al., 2012), Chilean propolis containing diarylheptanoids (Nina et al., 2015), triterpene-rich bee glue from Cameroon (Paul et al., 2014), to mention but a few.

4. Innovative phytochemical approaches to propolis analysis

Propolis is by definition a substance which contains compounds of powerful bioactivity, biosynthesized by plants for protection against pathogens and collected by bees for the same reason. Its healing properties have been known since antiquity. That is why the interest in propolis both as a medicine and as a source of new pharmacologically active chemical entities is steadily growing. The chemical analysis of propolis is important for both aspects of propolis research. Its use as a drug requires standardization, based on identification and quantification of the active principles. The search for new biologically active substances on the other hand relies on the detailed study of new propolis types from new botanical sources and unexplored regions and thus on the possibility of rapid and reliable dereplication of the chemical type of the particular sample.

A paradigm shift has occurred in natural product chemistry, in recent years. Up to the beginning of the 21st Century, plant specialized metabolites have been detected and analyzed by targeted methods for specific purposes, such as the quantification of bioactive plant metabolites, the evaluation of chemical variations in certain plants, etc. The development of powerful, modern analytical methods and chemometric data analysis has enabled the analysis of such metabolites in complex natural extracts by metabolic profiling and fingerprinting. Metabolite profiling concentrates on the analysis of a group of metabolites belonging to a particular compound class. Metabolic fingerprinting, on the other hand, compares patterns of metabolites without trying to identify individual, even major, metabolites. Both strategies can be used in the search for new biomarkers and can therefore be considered as complementary (Wolfender et al., 2013). When these analyses are performed on a large number of samples, they provide a basis for discrimination and classification of samples into groups. This type of holistic approach has been named metabolomics, and is considered as the large-scale phytochemical analysis of metabolites in a given object (Sumner et al., 2003), and it has found an application in propolis research.

Since 2007, the number of published propolis studies based on metabolomic and chemometric approaches has been increasing dramatically. Most of the investigations are aimed at classifying propolis samples into groups in order to determine their geographical origin and authenticity, and facilitate standardization.

Special attention has been paid to the assessment of the authenticity of Chinese poplar propolis and detection of poplar buds gum (balata) often used for its adulteration. Methods based on TLC chemometric fingerprinting (Tang et al., 2014), HPLC fingerprinting (Zhang and Hu, 2011), NIR and FT-NIR (Xu et al., 2013; Yang et al., 2016), and FT-IR combined with 2D-IR correlation spectroscopy and pattern recognition technique (Wu et al., 2008) were applied to solve this practical problem. TLC fingerprinting and pattern recognition methods have proved useful also for assessment of the authenticity of European poplar type propolis (Milojković-Opsenica et al., 2016).

Recognition of geographic origin was the aim of a number of studies based on different spectral methods and chemometrics. UV–vis reflectance spectral data and chemometric methods resulted in the assignment of Romanian samples into two groups according to their phytogeographic areas of collection: forests and meadows (Moţ et al., 2010). Ecosystem discrimination of Romanian propolis was achieved also by hierarchic fuzzy clustering and image analysis of TLC patterns (Sârbu and Moţ, 2011). HPLC fingerprints combined with similarity evaluation in simulative mean chromatograms among different regions could efficiently identify and distinguish Chinese propolis from different geographical origins (Zhou et al., 2008). Machine learning and chemometric-assisted interpretation of NMR data produced useful classification models for Brazilian propolis regarding its geographic origin (Maraschin et al., 2012).

In another study, HPLC fingerprints coupled with multivariate statistical analysis have been successfully applied to distinguish Chinese from Brazilian green propolis (Geng et al., 2016). However, it is not very clear why the authors had to apply chemometric approach to a problem which could be solved by a simple visual comparison of the HPLC chromatograms: in 2016, the chemical profiles and typical markers of these two propolis types had already been known for over 10 years.

GC-MS profiles of volatile propolis constituents combined with chemometrics were also used for geographic classification of propolis samples from China (Cheng et al., 2013) and from Brazil, Estonia, China

Table 2
Propolis plant sources, other than *Populus* spp.

Plant family	Plant species	Geographic origin	Marker compounds	References
Anacardiaceae	*Mangifera indica* L.	Myanmar	Alkylphenols, alkylresorcinols, anacardic acids, cycloartane triterpenes	Li et al., 2009
		Indonesia		Trusheva et al., 2011
		Oman		Popova et al., 2013
		Cameroon		Kardar et al., 2014
		Fiji		Trusheva et al., 2016
		Thailand		Sanpa et al., 2017
	Rhus javanica L. var. *chinensis* (Mill.) T.Yamaz (*Rhus chinensis* (Mill.)	Japan (Okayama)	No compounds identified, fingerprint comparison by HPLC	Murase et al., 2008
Apiaceae	*Angelica keskei* (Miq.) Koidz.	Korea, Jeju Island	Prenylated chalcones and coumarins	Shimomura et al., 2012
	Ferula communis L.	Malta	Terpenyl esters of substituted benzoic acid	Popova et al., 2011
	Ferula spp.	Iran	Terpenyl esters of substituted benzoic acid	Trusheva et al., 2010
Asteraceae	*Ambrosia deltoidea* (Torr.) Payne	USA, Sonora Desert	TLC fingerprint	Wollenweber and Buchmann, 1997
	Baccharis dracunculifolia DC	Brazil	C-prenylated cinnamic acids, caffeoylquinic acids	Kumazawa et al., 2003, Teixeira et al., 2005.
	Encelia farinosa Torr. & A.Gray	USA, Sonora Desert	TLC fingerprint	Wollenweber and Buchmann, 1997
	Psiadia punctulata (DC.) Vatke	Saudi Arabia	Flavonoid aglycones and diterpenes	Almutairi et al., 2014
Betulaceae	*Betula* spp.	Russia, Poland	Flavonoid aglycones, sesquiterpenyl esters of coumaric acid	Popravko, 1978; Isidorov et al., 2014
Cistaceae	*Cistus ladanifer* L.	Portugal (central and south parts)	Kaempferol methyl ethers	Falcão et al., 2013
Clusiaceae	*Clusia* spp.	Cuba, Venezuela, Brazil	Polyprenylated benzophenones	Tomás-Barberán et al., 1993; Cuesta Rubio et al., 1999
Cupressaceae	*Cupressus sempervirens* L.	Greek Mediterranean islands	Diterpenes of labdane and abietane type	Popova et al., 2010;
		Malta		Popova et al., 2012
Cyperaceae	*Lepidosperma* spp.	Australia, Kangaroo Island	Prenylated *p*-coumarate ester and stilbenes	Duke et al., 2017
Euphorbiaceae	*Macaranga tanarius* (L.) Müll.Arg.	Okinawa, Taiwan	Prenylated flavanones	Kumazawa et al., 2008
		Indonesia		Trusheva et al., 2011
		Hawaii		Inui et al., 2014
	Macaranga schweifurtii Pax	Kenya	Prenylated flavonoids, prenylated stilbenes	Petrova et al., 2010
	Macaranga spp.	Solomon Islands		Inui et al., 2012
		Fiji		Trusheva et al., 2016
Fabaceae	*Dalbergia ecastaphyllum* (L.) Taubert.	Brazil,	Isoflavonoids, pterocarpanes	Silva et al., 2008; Daugsch et al., 2008;
		Cuba		Piccinelli et al., 2011
	Dalbergia spp.	Nigeria		Omar et al., 2016
	Dalbergia spp.	Mexico		Lotti et al., 2010
	Dalbergia sisoo Roxb.	Nepal	Neoflavonoids	Awale et al., 2005a,b Shrestha et al., 2007
	Acacia nilotica (L.) Del.	Oman	Flavanes, pinitol	Popova et al., 2013
	Acacia paradoxa DC	Australia, Kangaroo Island	Methoxylated chalcones and flavonoid aglycones	Tran et al., 2012
	Mimosa tenuifolia L. (*Acacia tenuifolia* (L.) Willd.)	Brazil, Rio Grande do Norte State	Flavonols and chalcones	Ferreira et al., 2017
	Zuccagnia punctata Cav.	Argentina	Flavonoid aglycones, dihydrochalcones	Agüero et al., 2010; Salasa et al., 2016; Solorzano et al., 2017
Hamamelidaceae	*Liquidambar styraciflua* L.	Honduras	Cinnamic acid esters	Lotti et al., 2012
		USA, North Carolina		Savka et al., 2015
	Myroxylon balsamum (L.) Harms	El Salvador	benzyl benzoate, benzyl cinnamate	Popova et al., 2002
Lamiaceae	*Hyptis divaricata* Pohl ex Benth.	Brazil, Northeast parts	No compounds identified, fingerprint comparison by GC-MS and HPLC	Alencar et al., 2001
Meliaceae	*Azadirachta idnica* A.Juss.	Oman	C_5-prenyl flavanones	Popova et al., 2013
Scrophulariaceae	*Myoporum insulare* R.Br.	Southern Australia	Serrulatane diterpenes	Aminimoghadamfarouj and Nematollahi, 2017
Styracaceae	*Styrax* spp.	Thailand	cinnamic acid and benzoic acids esters	Athikomkulchai et al., 2013
Zygophyllaceae	*Larrea nitida* Cav.	Argentina, Andean region	Lignans	Agüero et al., 2011

and Uruguay (Kaškonienė et al., 2014). Even digital images of propolis samples (color models) plus chemometrics were successfully applied for indication of the geographical origin of samples from Buenos Aires Province (Pierini et al., 2016). In addition, several publications describe the multivariate analysis of mineral element concentration for grouping and prediction of the geographic origin of samples of Chinese (Gong et al., 2012) and Argentinean (Cantarelli et al., 2011) propolis.

UV–vis scanning spectrophotometry data sets were processed by chemometrics techniques and machine learning to allow discrimination of propolis samples from Southern Brazil according to the season of collection (Tomazzoli et al., 2015). NMR in combination with Factor Analysis and General Discriminant Analysis was used to classify Italian propolis obtained by different harvesting methods (Papotti et al., 2010).

It is important to note that all these studies include propolis samples from a relatively limited territorial range, and thus are of limited significance. To the best of our knowledge, none has actually been applied in industry. Moreover, information about the geographical origin of a sample does not necessarily give information about its chemical composition.

Metabolomic approaches have proved to be very useful in determining the plant origin of propolis. Metabolomic studies of poplar type propolis by HPTLC fingerprinting and multivariate analysis

(Ristivojević et al., 2014; Guzelmeric et al., 2018); ^{1}H and 2D J resolved NMR data and OPLS (Gođevac et al., 2016; Anđelković et al., 2017) and HPTLC-DART-MS (direct analysis with real time mass spectra) profiles combined with pattern recognition techniques clearly proved that samples from Serbia, Bulgaria, Macedonia, Turkey, Bosnia Herzegovina and France belong to two major types. The one from lower altitudes originates from *Populus nigra* buds, and the second one from higher altitudes (above 500–600 m) comes from aspen buds (*P. tremula*). The plant origin of propolis in Minnesota was revealed by metabolomic fingerprints generated using UPLC coupled to time-of-flight (TOF) mass spectrometry, compared to the fingerprints of different plant resins available to bees. Principal components analysis (PCA) was used to summarize metabolite patterns in the data and *P. deltoids* and *P. balsamifera* were identified as the preferred resin sources for bees (Wilson et al., 2013).

ESI-MS fingerprinting with PCA was used for fast and reliable characterization of propolis samples from different origins, and to determine the plant origins of the resins by comparing propolis extracts with extracts of possible plant sources (Sawaya et al., 2010). GC-MS of volatiles with PCA was also applied for source plant identification (Yan et al., 2012). Metabolomic approaches based on LC-MS revealed regional variations of propolis from the USA (Wilson et al., 2015) and the UK (Saleh et al., 2015), which pointed to differences in their botanical sources.

The metabolomic approach gives good results with respect to source identification however, it should be used carefully. Multivariate assisted digitally enhanced TLC analysis was utilized to determine the origin of Egyptian bee glue (Ibrahim and Metwally, 2017). Propolis was analyzed together with plant material, and the multivariate analysis pointed to *Eucalyptus globulus* as being the closest to propolis. However, a simple visual comparison of the published TLC demonstrates that propolis and eucalyptus extract have little in common, and it is highly improbable that eucalyptus is the main propolis source in the studied region.

Metabolomic approaches were applied in several studies aimed at creating rapid and simple procedures for determination of important quality parameters of propolis samples. UV–vis spectra and multivariate calibration model (PLS) allowed the determination of dry extract content in Brazilian commercial propolis extracts (Barbeira et al., 2013). ATR FT-IR combined with partial least square (PLS) regression was used for quantification of total phenolics and antioxidant activity (ABTS model) of Brazilian propolis from Parana State (Da Silva et al., 2018). Based on NIR spectra with PLS regression, a rapid method for quantification of total flavonoids in propolis from 4 Chinese provinces was developed (Cai et al., 2012). Once again, it should be stressed that these studies include propolis samples from a relatively limited territorial range and thus are of regional significance. They can hardly be applied to propolis samples from other locations without analysis of a new sample set.

Prediction of the antimicrobial activity of propolis samples from specific areas, such as Mexico and Libya was achieved based on multivariate analysis of antimicrobial and chemical profile data: ^{1}H-NMR (Rivero-Cruz et al., 2017) and LC-HRMS (Siheri et al., 2016). Again such results are valid only for propolis samples with similar chemical composition. A comparative study of the antibacterial activity of propolis samples from different geographic and climatic zones by means of PCA revealed that antibacterial activity was observed for samples from locations characterized by a wet-tropical rainforest-type climate (Seidel et al., 2008).

Analysis of data from biological tests and chemical profiles of propolis samples have helped to identify the active constituents of propolis. Correlation between LC-MS data and antimicrobial activity of South African propolis extracts by S-plot function identified chrysin, galangin, pinocembrin and pinobanksin-3-O-acetate as the bioactive constituents (Suleman et al., 2015). Partial least squares (PLS) modeling of the antitrypanosomal activity of Nigerian propolis extracts using the LC-MS data indicated that high activity could be correlated to denticulatain isomers in the extracts (Omar et al., 2016). Chemical profiling of propolis from healthy and infected colonies combined with PCA pointed to ferulic acid and coniferyl benzoate as potentially active substances against *Paenibacillus larvae* (Popova et al., 2017).

Metabolomic approaches can be useful in detection of new unstudied propolis types. Chemical profiling by different hyphenated techniques allowed dereplication of propolis samples from Oman (Popova et al., 2013), South Africa (Kasote et al., 2014) and sub-Saharan Africa (Zhang et al., 2014) and identification of new chemical types of propolis which yielded novel bioactive compounds.

It is interesting to mention here a study of the chemical characteristics of propolis types from Cuba, which used a large amount of chemical data collected by NMR, HPLC-PDA and HPLC-ESI/MS (Cuesta-Rubio et al., 2007). The authors did not apply chemometrics to their data set. Instead, they used their chemical knowledge and competences to define three chemical classes of Cuban propolis directly related to their specialized metabolite classes: brown Cuban propolis rich in polyisoprenylated benzophenones, red Cuban propolis containing isoflavonoids as the main constituents, and yellow Cuban propolis probably with aliphatic compounds. This approach delivered a basis for discrimination of the chemical types of propolis and in addition valuable information about their chemical constituents, which could not be directly obtained from chemometrics.

To conclude, metabolomics has great potential in propolis research but has to be applied with caution and the results of multivariate analyses should always be evaluated sticking to chemical common sense.

5. Problems of propolis standardization

Standardization of propolis is necessary for its application in different preparations, such as cosmetics, food additives, medical devices, etc. The most significant problem concerning propolis standardization is obviously the striking variability of its chemical composition, determined by the diverse source plants used by bees for resin collection/propolis production in different ecosystems. This chemical diversity creates a serious hindrance to the efforts for standardization and quality control and is one of the major reasons limiting the wider use of propolis in mainstream medical practice.

Labeling different propolis types as “just propolis” is the real problem, because although they all come from the beehive, they are the products of very different plant genomes. This is one of the major reasons why EFSA, the European Food Safety Authority, issued a scientific opinion (Agostoni et al., 2010) pronouncing non-compliance with the Regulation as on the basis of scientific evidence assessed, propolis containing products have been deemed not sufficiently characterized to be claiming an effect on a health condition and the claim could not therefore be substantiated.

How could this problem be solved? Bee glue is a plant derived product and it has been proven that bees do not change the chemical composition of plant resins. Therefore, it is completely reasonable to address the problem of propolis standardization in the same way as is done for medicinal plants: based on biologically active specialized metabolites. However, just a look at Table 2., summarizing new propolis plant sources, makes it clear that a universal solution is impossible. Trying to develop universal standardization parameters for propolis would mean attempting a universal standardization for plant extracts, derived from plant species belonging to different plant families. While no one has ever taken such an initiative for plants, because it obviously does not make sense, the attempts to standardize propolis regardless its chemical type have been numerous.

To overcome this obstacle, it is necessary to classify different propolis types according to their plant source and the corresponding chemical profile, and standardize them as different natural products - specific types of propolis., e.g. as “Poplar type propolis” or “Green

Brazilian propolis", not as "propolis". Bee glue could be easily characterized based on its plant source. The resin of the source plant(s) with its genetically foreordained chemical composition determines the chemistry of the respective propolis. As long as the composition of the corresponding plant exudates is known, this method gives information about the qualitative composition of the sample and the compounds which have to be quantified.

Even if the approach to standardization based on the chemical type of propolis is adopted, the complex composition of any particular propolis chemical type poses an additional challenge to the analytical efforts. A significant number of analytical procedures have been reported, aimed at quantifying individual propolis constituents. These procedures were based on different methods: GC-MS, LC-MS, HPLC, and NMR (review: Bankova et al., 2016). However, especially concerning antibacterial activity, no individual component has demonstrated an activity higher than that of propolis total extract. Some authors studied correlations between the MIC and concentrations of various active propolis components and found that no individual compound surpassed Pearson-Lee values (Bonvehi et al., 1994). Synergism between propolis constituents has also been observed (Amoros et al., 1992; Santos et al., 2002a,b; Kosalec et al., 2003). For this reason, holistic approaches to quantitative analysis of bioactive constituents should be appropriate.

Taking into account the fact that known propolis types contains dozens of individual constituents, most of them with known biological activity, the quantification of groups of bioactive compounds was suggested. Phenolic compounds and flavonoids, which are characteristic for several propolis types, can be easily and rapidly determined as total phenolics and total flavonoids by simple spectrophotometric methods. Such methods have been applied by many researchers to propolis samples from different locations all over the world but there are several critical points, especially when using the Folin-Ciocalteu (FC) method for quantification of total phenolics. The FC reagent-based assay have gained popularity and is commonly known as the total phenols (or phenolic) assay. The FC actually measures a sample's reducing capacity, not the real amount of phenolic compounds in the sample, if measured as gallic acid equivalents. This fact is not reflected in the name "total phenolic assay". For this reason it is often misunderstood. Numerous publications have applied the total phenolics assay by FC reagent and an other electron transfer-based antioxidant capacity assay (e.g., FRAP, DPPH, etc.) and often found excellent linear correlations between the "total phenolic profiles" and "the antioxidant activity". This is not surprising if one considers the similarity of chemistry between the two assays. One of the assays may just be redundant (Huang et al., 2005).

For this reason, efforts were directed towards finding a calibration standard appropriate for the specific phenolic profile of poplar type propolis, which could give the real quantity of the sum of phenolic compounds (Popova et al., 2004). Taking into account reliable data about the amount of major poplar propolis constituents, a model mixture of 14 propolis constituents was prepared, including phenolic acids and their esters, flavanones, dihydroflavonols, flavones and flavonols, and applied this mixture for method validation. Using this approach, it became clear that neither gallic acid nor caffeic acid appeared to be suitable references because of the low recovery established (about 50%). Obviously, another reference calibration standard was necessary for the analysis of poplar-type propolis. After a series of experiments, a mixture of a flavanone and a flavonol, pinocembrin and galangin 2:1 provided acceptable results (over 90% recovery) Thus, the real amount of phenolics in poplar type propolis was determined. To complete the quantitative characterization of poplar type propolis, total flavones/flavonols by the $AlCl_3$ method and flavanones dihydroflavonols by the dinitrophenyl hydrazine (DNP) colorimetric method were quantified separately. A separate procedure for flavanones is necessary because they are important constituents of poplar type propolis but cannot be properly quantified by the $AlCl_3$ method, because the absorption maxima of flavanone aluminium complexes are very different from those of flavones. The results were verified independently by an HPLC procedure. Applying the combination of spectrophotometric methods to over 100 poplar propolis samples from different geographic origins, significant negative correlation between the amount of total phenolics and MIC was observed (Popova et al., 2007). Thus, the measurement of total phenolics is an important, reliable criterion of quality for poplar propolis. The International Honey Commission has accepted the abovementioned methods and recommends them for standardization purposes of poplar type propolis (Bankova et al., 2016). A similar approach was applied also for Pacific type propolis for quantification of propolins (prenylated flavanones) as total flavanones by the DNP methods, using a defined mixture of propolins as the calibration standard.

Of course, chromatographic and hyphenated techniques can also be very useful in standardization and quality control. When fingerprinting by such methods is used for standardization purposes, the first task of the analysis should be identification of the chemical type of propolis. Quantification should be focused on a large number of specialized metabolites, not on one or a couple of active compounds.

The most important aspect of solving the problem of propolis standardization remains the understanding that any standard and quality control procedure, based on concentration of active principles should be directed to one particular well-defined chemical propolis type.

6. Prospects and conclusions

Honeybees and flowering plants are involved in a complex mutualistic relationship. Specialized plant metabolites play an important role in this relationship: volatiles are olfactory cues attracting the bees as pollinators (Pham-Delegue et al., 1987); visual color patterns based mainly on different types of flavonoids are also attractive to bees (Wester and Lunau, 2017); numerous specialized metabolites are present in the hive as constituents of the plant materials used by bees as food: pollen and nectar/honey, and as building/protective material: propolis. Thus, the beehive offers a unique view into phytochemicals and their importance for bee-plant interaction. The significance of specialized plant metabolites in the defense of the colony from pathogenic microorganisms and parasites is yet to be revealed in detail.

Propolis, as a defensive substance very rich in specialized metabolites, has received a lot of attention from phytochemists and pharmacologists. In addition, propolis is the plant-derived bee product with most potent and diverse biological activities and a large number of studies have been directed to the investigation of these activities. However, a significant proportion of these studies are yet published without any chemical study of the propolis used. This fact is most unfortunate because among them, there are investigations of high quality, regarding the pharmacological part, and also those that report valuable activities (Bankova and Trusheva, 2013). The understanding that the term "propolis" does not have any chemical connotations, unlike the scientific name of a plant species, has lead to the paradigm shift in the propolis research which was completed in the last decade. It is vital to stress that any research of propolis' pharmacological effects without chemical characterization is irreproducible and irrelevant, practically useless and thus a waste of time and effort.

In conclusion, the phytochemistry of honeybees is an exciting research field with the potential to reveal new ecological relationships between plants and bees, new chemical structures, new biologically active compounds and new applications of the well-known products. Plant derived bee products remain a fascinating subject for further study, and we can expect more surprises that bees have prepared for us.

Acknowledgements

Financial support by the Bulgarian Academy of Sciences is gratefully acknowledged.

References

Adelmann, J., Passos, M., Breyer, D.H., dos Santos, M.H.R., Lenz, C., Leite, N.F., Lanças, F.M., Fontana, J.D., 2007. Exotic flora dependence of an unusual Brazilian propolis: the pinocembrin biomarker by capillary techniques. J. Pharmaceut. Biomed. Anal. 43 (1), 174–178.

Agostoni, C., Bresson, J., Fairweather-Tait, S., Flynn, A., Golly, I., Korhonen, H., Lagiou, P., Løvik, M., Marchelli, R., Martin, A., Moseley, B., Neuhäuser-Berthold, M., Przyrembel, H., Salminen, S., Sanz, Y., Strain, S., Strobel, S., Tetens, I., Tomé, D., van Loveren, H., Verhagen, H., 2010. Scientific opinion on the substantiation of health claims related to propolis ID 1242, 1245, 1246, 1247, 1248, 3184 and flavonoids in propolis ID 1244, 1644, 1645, 3526, 3527, 3798, 3799 pursuant to Article 13 1 of Regulation EC No 1924/2006. EFSA J. 810, 1810.

Agüero, M.B., Gonzalez, M., Lima, B., Svetaz, L., Sánchez, M., Zacchino, S., Feresin, G.E., Schmeda-Hirschmann, G., Palermo, J., Wunderlin, D., Tapia, A., 2010. Argentinean propolis from *Zuccagnia punctata* Cav.(Caesalpinieae) exudates: phytochemical characterization and antifungal activity. J. Agric. Food Chem. 58 (1), 194–201.

Agüero, M.B., Svetaz, L., Sánchez, M., Luna, L., Lima, B., López, M.L., Zacchino, S., Palermo, J., Wunderlin, D., Feresin, G.E., Tapia, A., 2011. Argentinean Andean propolis associated with the medicinal plant *Larrea nitida* Cav.(Zygophyllaceae). HPLC–MS and GC–MS characterization and antifungal activity. Food Chem. Toxicol. 49 (9), 1970–1978.

Alencar, S.M., Park, Y.K., Aguiar, C.L., Mariuzzo, D.M., 2001. Evidências fitoquímicas da própolis da região de mata atlântica do estado da Bahia. Tecbahia Revista Baiana De Tecnologia 16, 154–160.

Awale, S., Shrestha, S.P., Tezuka, Y., Ueda, J.Y., Matsushige, K., Kadota, S., 2005a. Neoflavonoids and related constituents from Nepalese propolis and their nitric oxide production inhibitory activity. J. Nat. Prod. 68 (6), 858–864.

Alfonsus, E.C., 1933. Some sources of propolis. Glean. Bee Cult. 61, 92–93.

Aliboni, A., 2014. Propolis from Northern California and Oregon: chemical composition, botanical origin, and content of allergens. Z. Naturforsch., C: Biosci. 69 (1–2), 10–20.

Almutairi, S., Edrada-Ebel, R., Fearnley, J., Igoli, J.O., Alotaibi, W., Clements, C.J., Gray, A.I., Watson, D.G., 2014. Isolation of diterpenes and flavonoids from a new type of propolis from Saudi Arabia. Phytochem. Lett. 10, 160–163.

Alqarni, A.S., Rushdi, A.I., Owayss, A.A., Raweh, H.S., El-Mubarak, A.H., Simoneit, B., 2015. Organic tracers from asphalt in propolis produced by urban honey bees, *Apis mellifera* Linn. PLoS One 106 e0128311.

Aminimoghadamfarouj, N., Nematollahi, A., 2017. Structure elucidation and botanical characterization of diterpenes from a specific type of bee glue. Molecules 227, 1185.

Amoros, M., Simõs, C.M.O., Girre, L., Sauvager, F., Cormier, M., 1992. Synergistic effect of flavones and flavonols against herpes simplex virus type 1 in cell culture. Comparison with the antiviral activity of propolis. J. Nat. Prod. 55 (12), 1732–1740.

Anđelković, B., Vujisić, L., Vučković, I., Tešević, V., Vajs, V., Gođevac, D., 2017. Metabolomics study of *Populus* type propolis. J. Pharmaceut. Biomed. Anal. 135, 217–226.

Ares, A.M., Valverde, S., Bernal, J.L., Nozal, M.J., Bernal, J., 2018. Extraction and determination of bioactive compounds from bee pollen. J. Pharmaceut. Biomed. Anal. 147, 110–124.

Athikomkulchai, S., Awale, S., Ruangrungsi, N., Ruchirawat, S., Kadota, S., 2013. Chemical constituents of Thai propolis. Fitoterapia 88, 6–100.

Awale, S., Shrestha, S.P., Tezuka, Y., Ueda, J., Matsushige, K., Kadota, S., 2005b. Neoflavonoids and related constituents from Nepalese propolis and their nitric oxide production inhibitory activity. J. Nat. Prod. 68, 858–864.

Bankova, V., Bertelli, D., Borba, R., Conti, B.J., da Silva Cunha, I.B., Danert, C., Eberlin, M.N., Falcão, S.I., Isla, M.I., Nieva Moreno, M.I., Papotti, G., Popova, M., Basso Santiago, K., Salas, A., Frankland Sawaya, A.C.H., Schwab, N.V., Sforcin, J.M., Simone-Finstrom, M., Spivak, M., Trusheva, B., Vilas-Boas, M., Wilson, M., Zampini, C., 2016. Standard methods for *Apis mellifera* propolis research. J. Apicult. Res. https://doi.org/10.1080/00218839.2016.1222661.

Bankova, V., de Castro, S.L., Marcucci, M.C., 2000. Propolis: recent advances in chemistry and plant origin. Apidologie 31, 3–15.

Bankova, V., Popova, M., Trusheva, B., 2006. Plant sources of propolis: an update from a chemist's point of view. Nat. Prod. Commun. 1 (11), 1023–1028.

Bankova, V., Popova, M., Trusheva, B., 2014. Propolis volatile compounds: chemical diversity and biological activity: a review. Chem. Cent. J. 8 (1), 28.

Bankova, V., Trusheva, B., 2013. Chemical profiles of different propolis types in relation to their biological activity, in: Farooki, T., Farooki, A. (Eds.), Beneficial Effects of Propolis on Human Health and Chronic Diseases. NOVA Science Publishers, New York, pp. 1 – 38.

Barbeira, P.J., Paganotti, R.S., Ássimos, A.A., 2013. Development of a multivariate calibration model for the determination of dry extract content in Brazilian commercial bee propolis extracts through UV–Vis spectroscopy. Spectrochim. Acta, Part A 114, 441–448.

Barth, O.M., da Luz, C.F.P., 2009. Palynological analysis of Brazilian red propolis samples. J. Apicult. Res. 48 (3), 181–188.

Bertrams, J., Kunz, N., Müller, M., Kammerer, D., Stintzing, F.C., 2013. Phenolic compounds as marker compounds for botanical origin determination of German propolis samples based on TLC and TLC-MS. J. Appl. Bot. Food Qual. 86, 143–153.

Bonvehi, J.S., Coll, F.V., Jorda, R.E., 1994. The composition, active components and bacteriostatic activity of propolis in dietetics. J. Am. Oil Chem. Soc. 71, 529–532.

Burdock, G.A., 1998. Review of the biological properties and toxicity of bee propolis propolis. Food Chem. Toxicol. 364, 347–363.

Cai, R., Wang, S., Meng, Y., Meng, Q., Zhao, W., 2012. Rapid quantification of flavonoids in propolis and previous study for classification of propolis from different origins by using near infrared spectroscopy. Anal. Meth. 48, 2388–2395.

Cantarelli, M.Á., Camiña, J.M., Pettenati, E.M., Marchevsky, E.J., Pellerano, R.G., 2011. Trace mineral content of Argentinean raw propolis by neutron activation analysis NAA: assessment of geographical provenance by chemometrics. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 44 (1), 256–260.

Castro-Falcón, R., Pulido-Ávila, M.G., Muñoz-Urías, A., Islas-Rodríguez, A.E., 2016. Antimicrobial activity and palynological characterization of propolis samples collected in Mexico over the course of one year. Rev. Latinoam. Quim. 44 17-16.

Çelemli, Ö.G., Temizer, İ.K., Zare, G., Sorkun, K., 2016. *Castanea sativa*; a source of Turkish propolis: plant anatomy, palynology and chemistry. Hacettepe J. Biol. Chem. 44 (1), 7–14.

Chalcoff, V.R., Aizen, M.A., Galetto, L., 2005. Nectar concentration and composition of 26 species from the temperate forest of South America. Ann. Bot. 97 (3), 413–421.

Cheng, H., Qin, Z.H., Guo, X.F., Hu, X.S., Wu, J.H., 2013. Geographical origin identification of propolis using GC–MS and electronic nose combined with principal component analysis. Food Res. Int. 51 (2), 813–822.

Christov, R., Trusheva, B., Popova, M., Bankova, V., Bertrand, M., 2006. Chemical composition of propolis from Canada, its antiradical activity and plant origin. Nat. Prod. Res. 20 (06), 531–536.

Crane, E., 1990. Bees and Beekeeping: Science, Practice and World Resources, Heinemann Newnes, Oxford.

Cuesta-Rubio, O., Cuellar, A.C., Rojas, N., Castro, H.V., Rastrelli, L., Aquino, R., 1999. A polyisoprenylated benzophenone from Cuban propolis. J. Nat. Prod. 62 (7), 1013–1015.

Cuesta-Rubio, O., Piccinelli, A.L., Campo Fernandez, M., Marquez Hernandez, I., Rosado, A., Rastrelli, L., 2007. Chemical characterization of Cuban propolis by HPLC− PDA, HPLC− MS, and NMR: the brown, red, and yellow Cuban varieties of propolis. J. Agric. Food Chem. 55 (18), 7502–7509.

Da Silva, C., Prasniewski, A., Calegari, M.A., deLima, V.A., Oldon, T.L.C., 2018. Determination of total phenolic compounds and antioxidant activity of ethanolic extracts of propolis using ATR–FT-IR spectroscopy and chemometrics. Food Anal. Meth. https://doi.org/10.1007/s12161-018-1161-x.

Daugsch, A., Moraes, C.S., Fort, P., Park, Y.K., 2008. Brazilian red propolis—chemical composition and botanical origin. Evid. Compl. Alter. Med. 5 (4), 435–441.

De Freitas, A.D.S., Barth, O.M., de Oliveira Sales, É., Matsuda, A.H., de Almeida-Muradian, L.B., 2011. A palynological analysis of Brazilian propolis samples. J. ApiProd ApiMed. Sci. 3 (2), 67–74.

De-Melo, A. A. M., de Almeida-Muradian, L. B., 2017. Chemical composition of bee pollen, in: Alvarez-Suarez, J. M. (Ed.), Bee Products-chemical and Biological Properties. Springer, Cham, pp. 221-259.

Dias, L.G., Pereira, A.P., Estevinho, L.M., 2012. Comparative study of different Portuguese samples of propolis: pollinic, sensorial, physicochemical, microbiological characterization and antibacterial activity. Food Chem. Toxicol. 50 (12), 4246–4253.

Duke, C.C., Tran, V.H., Duke, R.K., Abu-Mellal, A., Plunkett, G.T., King, D.I., Hamid, K., Wilson, K.L., Barret, R.L., Bruhl, J.J., 2017. A sedge plant as the source of Kangaroo Island propolis rich in prenylated p-coumarate ester and stilbenes. Phytochemistry 134, 87–97.

Erler, S., Moritz, R.F., 2016. Pharmacophagy and pharmacophory: mechanisms of self-medication and disease prevention in the honeybee colony *Apis mellifera*. Apidologie 47 (3), 389–411.

Falcão, S.I., Vale, N., Gomes, P., Domingues, M.R., Freire, C., Cardoso, S.M., Vilas-Boas, M., 2013. Phenolic profiling of Portuguese propolis by LC–MS spectrometry: uncommon propolis rich in flavonoid glycosides. Phytochem. Anal. 24 (4), 309–318.

Ferreira, J.M., Fernandes-Silva, C.C., Salatino, A., Negri, G., Message, D., 2017. New propolis type from north-east Brazil: chemical composition, antioxidant activity and botanical origin. J. Sci. Food Agric. 97 (11), 3552–3558.

Geng, L., Meng, F., Zhao, M., Wang, L., Liu, Y., Wang, L., 2016. Discrimination of Brazilian green propolis and Chinese propolis based on high-performance liquid chromatographic fingerprints and multivariate statistical analysis. J. Integr. Med. 64 (Suppl. 1), A16. http://jim.bmj.com/content/64/Suppl_8/A6.1.

Gođevac, D., Anđelković, B., Vajs, V., Tešević, V., 2016. The effects of altitude on the chemical composition of *Populus* type propolis. Planta Med. 82 (S 01), P215.

Gong, S., Luo, L., Gong, W., Gao, Y., Xie, M., 2012. Multivariate analyses of element concentrations revealed the groupings of propolis from different regions in China. Food Chem. 134 (1), 583–588.

Greenaway, W., Scaysbrook, T., Whatley, F.R., 1990. The composition and plant origins of propolis: a report of work at Oxford. Bee World 71 (3), 107–118.

Guzelmeric, E., Ristivojević, P., Trifković, J., Dastan, T., Yilmaz, O., Cengiz, O., Yesilada, E., 2018. Authentication of Turkish propolis through HPTLC fingerprints combined with multivariate analysis and palynological data and their comparative antioxidant activity. LWT-Food Sci. Technol. 87, 23–32.

Hu, H., Dong, J., Zhang, H., Zeng, X.-X., 2014. Behavior observation of worker bees collecting propolis and the plant origin of propolis. Food Sci. 35 (15), 54–58 [in Chinese].

Huang, D., Ou, B., Prior, R.L., 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53 (6), 1841–1856.

Huang, S., Zhang, C.P., Li, G.Q., Sun, Y.Y., Wang, K., Hu, F.L., 2014. Identification of catechol as a new marker for detecting propolis adulteration. Molecules 19 (7), 10208–10217.

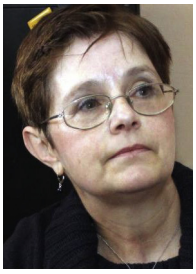
Ibrahim, R.S., Metwally, A., 2017. M. Plant source of Egyptian propolis using multivariate-assisted digitally-enhanced TLC data analysis. J. Nat. Prod. Plant Resour. 73, 20–25.

Inui, S., Hosoya, T., Kumazawa, S., 2014. Hawaiian propolis: comparative analysis and botanical origin. Nat. Prod. Commun. 9 (2), 165–166.

Inui, S., Hosoya, T., Shimamura, Y., Masuda, S., Ogawa, T., Kobayashi, H., Shirafuji, K., Toli Moli, R., Kozone, I., Shin-ya, K., Kumazawa, S., 2012. Solophenols B–D and solomonin: new prenylated polyphenols isolated from propolis collected from the

Solomon Islands and their antibacterial activity. J. Agric. Food Chem. 60 (47), 11765–11770.
Isidorov, V.A., Bakier, S., Pirożnikow, E., Zambrzycka, M., Swiecicka, I., 2016. Selective behaviour of honeybees in acquiring European propolis plant precursors. J. Chem. Ecol. 42 (6), 475–485.
Isidorov, V.A., Szczepaniak, L., Bakier, S., 2014. Rapid GC/MS determination of botanical precursors of Eurasian propolis. Food Chem. 142, 101–106.
Isla, M.I., Paredes-Guzman, J.F., Nieva-Moreno, M.I., Koo, H., Park, Y.K., 2005. Some chemical composition and biological activity of northern Argentine propolis. J. Agric. Food Chem. 53 (4), 1166–1172.
Kaczmarek, F., Dębowski, W.J., 1983. Występowanie α- i β-amylazy w propolisie. Acta Polon. Pharm. 40, 121.
Kardar, M.N., Zhang, T., Coxon, G.D., Watson, D.G., Fearnley, J., Seidel, V., 2014. Characterisation of triterpenes and new phenolic lipids in Cameroonian propolis. Phytochemistry 106, 156–163.
Kaškonienė, V., Kaškonas, P., Maruška, A., Kubilienė, L., 2014. Chemometric analysis of volatiles of propolis from different regions using static headspace GC-MS. Cent. Eur. J. Chem. 12 (6), 736–746.
Kasote, D., Suleman, T., Chen, W., Sandasi, M., Viljoen, A., van Vuuren, S., 2014. Chemical profiling and chemometric analysis of South African propolis. Biochem. Syst. Ecol. 55, 156–163.
Kędzia, B., 2011. Chemical composition of Polish propolis. Part I. the initial period of investigations. Postępy Fitoterapii 10 (1), 39–44.
Kosalec, I., Bakmaz, M., Pepeljnjak, S., 2003. Analysis of propolis from continental and Adriatic region of Croatia. Acta Pharm. Zagreb 53 (4), 275–286.
Kumazawa, S., Ahn, M.R., Fujimoto, T., Kato, M., 2010. Radical-scavenging activity and phenolic constituents of propolis from different regions of Argentina. Nat. Prod. Res. 24 (9), 804–812.
Kumazawa, S., Nakamura, J., Murase, M., Miyagawa, M., Ahn, M.R., Fukumoto, S., 2008. Plant origin of Okinawan propolis: honeybee behavior observation and phytochemical analysis. Naturwissenschaften 95 (8), 781–786.
Kumazawa, S., Yoneda, M., Shibata, I., Kanaeda, J., Hamasaka, T., Nakayama, T., 2003. Direct evidence for the plant origin of Brazilian propolis by the observation of honeybee behavior and phytochemical analysis. Chem. Pharm. Bull. 51 (6), 740–742.
Lavie P., 1976. The relationship between propolis, poplar buds *Populus* spp. and castoreum, Proc. XXV Int. Beekeeping Congr., Grenoble, 1975, Apimondia Publ. House, Bucharest, pp. 229–233.
Leonhardt, S.D., Zeilhofer, S., Blüthgen, N., Schmitt, T., 2010. Stingless bees use terpenes as olfactory cues to find resin sources. Chem. Senses 35, 603–611.
Li, F., Awale, S., Tezuka, Y., Esumi, H., Kadota, S., 2010. Study on the constituents of Mexican propolis and their cytotoxic activity against PANC-1 human pancreatic cancer cells. J. Nat. Prod. 73 (4), 623–627.
Li, F., Awale, S., Zhang, H., Tezuka, Y., Esumi, H., Kadota, S., 2009. Chemical constituents of propolis from Myanmar and their preferential cytotoxicity against a human pancreatic cancer cell line. J. Nat. Prod. 72 (7), 1283–1287.
Lotti, C., Campo Fernandez, M., Piccinelli, A.L., Cuesta-Rubio, O., Márquez Hernández, I., Rastrelli, L., 2010. Chemical constituents of red Mexican propolis. J. Agric. Food Chem. 58 (4), 2209–2213.
Lotti, C., Piccinelli, A.L., Arevalo, C., Ruiz, I., Migliani De Castro, G.M., Figueira Reis De Sá, L., Tessis, A.C., Ferreira-Pereira, A., Rastrelli, L., 2012. Constituents of Hondurian propolis with inhibitory effects on *Saccharomyces cerevisiae* multidrug resistance protein Pdr5p. J. Agric. Food Chem. 60 (42), 10540–10545.
Maraschin, M., Somensi-Zeggio, A., Oliveira, S. K., Kuhnen, S., Tomazzoli, M. M., Zeri, A. C., Carreira, R. Rocha, M., 2012, A machine learning and chemometrics assisted interpretation of spectroscopic data–a NMR-based metabolomics platform for the assessment of Brazilian propolis, in: Shibuya, T., Kashima, H., Sese, J., Ahmad, S. (Eds.) Pattern Recognition in Bioinformatics. PRIB 2012. Lecture Notes in Computer Science, vol vol. 7632. Springer, Berlin, Heidelberg, pp. 129-140.
Markham, K.R., Mitchell, K.A., Wilkins, A.L., Daldy, J.A., Lu, Y., 1996. HPLC and GC-MS identification of the major organic constituents in New Zealand propolis. Phytochemistry 42 (1), 205–211.
Matos, V.R., dos Santos, F.D.A.R., 2017. The pollen spectrum of the propolis of *Apis mellifera* L. Apidae from the Atlantic Rainforest of Bahia. Braz. Palynol. 41 (2), 207–215.
Meyer, W., 1956. “Propolis bees” and their activities. Bee World 37 (2), 25–36.
Milojković Opsenica, D., Ristivojević, P., Trifković, J., Vovk, I., Lušić, D., Tešić, Ž., 2016. TLC fingerprinting and pattern recognition methods in the assessment of authenticity of poplar-type propolis. J. Chromatogr. Sci. 54 (7), 1077–1083.
Morse, G., 1975. Über Propolis und ihre Verwendung im Bienenvolk. In: Die Propolis. Apimondia Bukarest, pp.11-15.
Moţ, A.C., Soponar, F., Sârbu, C., 2010. Multivariate analysis of reflectance spectra from propolis: geographical variation in Romanian samples. Talanta 81 (3), 1010–1015.
Murase, M., Kato, M., Sun, A., Ono, T., Nakamura, J., Sato, T., Kumazawa, S., 2008. *Rhus javanica* var. *chinensis* as a new plant origin of propolis from Okayama, Japan. Biosci. Biotech. Biochem. 72 (10), 2782–2784.
Negri, G., Marcucci, C., Salatino, A., Salatino, M.L.F., 2000. Comb and propolis waxes from Brazil (States of São Paulo and Paraná). J. Braz. Chem. Soc. 11 (5), 453–457.
Nina, N., Quispe, C., Jiménez-Aspee, F., Theoduloz, C., Feresín, G.E., Lima, B., … Schmeda-Hirschmann, G., 2015. Antibacterial activity, antioxidant effect and chemical composition of propolis from the Región del Maule, central Chile. Molecules 20 (10), 18144–18167.
Omar, R., Igoli, J.O., Zhang, T., Gray, A.I., Ebiloma, G.U., Clements, C.J., Fearnley, J., Edrada-Ebel, R.A., Paget, T., de Koning, H.P., Watson, D.G., 2016. The chemical characterization of Nigerian propolis samples and their activity against *Trypanosoma brucei*. Sci. Rep. 7, 923. https://doi.org/10.1038/s41598-017-01038-2.
Papotti, G., Bertelli, D., Plessi, M., Rossi, M.C., 2010. Use of HR-NMR to classify propolis obtained using different harvesting methods. Int. J. Food Sci. Technol. 45 (8), 1610–1618.
Pasupuleti, V.R., Sammugam, L., Ramesh, N., Gan, S.H., 2017. Honey, propolis, and royal jelly: a comprehensive review of their biological actions and health benefits. Oxid. Med. Cell. Long. 2017, 1259510 21 pages.
Paul, S., Emmanuel, T., Matchawe, C., Alembert, T.T., Elisabeth, Z.O.M., Sophie, L., Luce, V.E., Maurice, T.R., Abel Joel, Y.G., De Théodore Alex, A., Joseph, M.T., 2014. Pentacyclic triterpenes and crude extracts with antimicrobial activity from Cameroonian brown propolis samples. J. Appl. Pharmaceut. Sci. 4 (07), 001–009.
Petrova, A., Popova, M., Kuzmanova, C., Tsvetkova, I., Naydenski, H., Muli, E., Bankova, V., 2010. New biologically active compounds from Kenyan propolis. Fitoterapia 81 (6), 509–514.
Pham-Delegue, M.H., Etievant, P., Masson, C., 1987. Molecular parameters involved in bee-plant relationships: a biological and chemical approach. Biochimie 69 (6–7), 661–670.
Piccinelli, A.L., Lotti, C., Campone, L., Cuesta-Rubio, O., Campo Fernandez, M., Rastrelli, L., 2011. Cuban and Brazilian red propolis: botanical origin and comparative analysis by high-performance liquid chromatography–photodiode array detection/electrospray ionization tandem mass spectrometry. J. Agric. Food Chem. 59 (12), 6484–6491.
Piccinelli, A.L., Mencherini, T., Celano, R., Mouhoubi, Z., Tamendjari, A., Aquino, R.P., Rastrelli, L., 2013. Chemical composition and antioxidant activity of Algerian propolis. J. Agric. Food Chem. 61 (21), 5080–5088.
Pierini, G.D., Fernandes, D.D.S., Diniz, P.H.G.D., de Araújo, M.C.U., Di Nezio, M.S., Centurión, M.E., 2016. A digital image-based traceability tool of the geographical origins of Argentine propolis. Microchem. J. 128, 62–67.
Popova, M.P., Bankova, V.S., Bogdanov, S., Tsvetkova, I., Naydenski, C., Marcazzan, G.L., Sabatini, A.G., 2007. Chemical characteristics of poplar type propolis of different geographic origin. Apidologie 38 (3), 306–311.
Popova, M.P., Graikou, K., Chinou, I., Bankova, V.S., 2010. GC-MS profiling of diterpene compounds in Mediterranean propolis from Greece. J. Agric. Food Chem. 58 (5), 3167–3176.
Popova, M., Antonova, D., Bankova, V., 2017. Chemical composition of propolis and American foulbrood: is there any relationship? Bulg. Chem. Commun. 49 (Special Issue D), 171–175.
Popova, M., Bankova, V., Butovska, D., Petkov, V., Nikolova-Damyanova, B., Sabatini, A.G., Marcazzan, G.L., Bogdanov, S., 2004. Validated methods for the quantification of biologically active constituents of poplar-type propolis. Phytochem. Anal. 154, 235–240.
Popova, M., Bankova, V., Chimov, A., Silva, M.V., 2002. A scientific note on the high toxicity of propolis that comes from *Myroxylon balsamum* trees. Apidologie 33 (1), 87–88.
Popova, M., Dimitrova, R., Al-Lawati, H.T., Tsvetkova, I., Najdenski, H., Bankova, V., 2013. Omani propolis: chemical profiling, antibacterial activity and new propolis plant sources. Chem. Cent. J. 7 (1), 158.
Popova, M., Trusheva, B., Antonova, D., Cutajar, S., Mifsud, D., Farrugia, C., Tsvetkova, I., Najdenski, H., Bankova, V., 2011. The specific chemical profile of Mediterranean propolis from Malta. Food Chem. 126 (3), 1431–1435.
Popova, M., Trusheva, B., Cutajar, S., Antonova, D., Mifsud, D., Farrugia, C., Bankova, V., 2012. Identification of the plant origin of the botanical biomarkers of Mediterranean type propolis. Nat. Prod. Commun. 7 (5), 569–570.
Popravko S.A., 1978. Chemical composition of propolis, its origin and standardization, in: A Remarkable Hive Product: PROPOLIS, Apimondia Publ. House, Bucharest, , pp. 15–18.
Popravko, S.A., Sokolov, M.V., 1980. Plant sources of propolis. Pchelovodstvo 2, 28–29 [in Russian].
Ristivojević, P., Andrić, F.L., Trifković, J.Đ., Vovk, I., Stanisavljević, L.Ž., Tešić, Ž.L., Milojković-Opsenica, D.M., 2014. Pattern recognition methods and multivariate image analysis in HPTLC fingerprinting of propolis extracts. J. Chemom. 28 (4), 301–310.
Rivero-Cruz, J.F., Rodríguez de San Miguel, E., Robles-Obregón, S., Hernández-Espino, C.C., Rivero-Cruz, B.E., Pedraza-Chaverri, J., Esturau-Escofet, N., 2017. Prediction of antimicrobial and antioxidant activities of Mexican propolis by 1H-NMR spectroscopy and chemometrics data analysis. Molecules 22 (7), 1184.
Salasa, A., Mercadoc, M.I., Zampini, I.C., Ponessa, G.I., Isla, M.I., 2016. Determination of botanical origin of propolis from Monte region of Argentina by histological and chemical methods. Nat. Prod. Commun. 11 (5), 627–630.
Salatino, A., Fernandes-Silva, C.C., Righi, A.A., Salatino, M.L.F., 2011. Propolis research and the chemistry of plant products. Nat. Prod. Rep. 28 (5), 925–936.
Salatino, A., Salatino, M.L.F., 2017. Why do honeybees exploit so few plant species as propolis sources? MOJ Food Process. Technol. 45, 00107. https://doi.org/10.15406/mojfpt.2017.04.00107.
Salatino, A., Weinstein Teixeira, W., Negri, G., Message, D., 2005. Origin and chemical variation of Brazilian propolis. Evid. Compl. Alter. Med. 2, 33–38.
Saleh, K., Zhang, T., Fearnley, J., Watson, D.G., 2015. A comparison of the constituents of propolis from different regions of the United Kingdom by liquid chromatography-high resolution mass spectrometry using a metabolomics approach. Curr. Metabol. 3 (1), 42–53.
Sanpa, S., Popova, M., Tunkasiri, T., Eitssayeam, S., Bankova, V., Chantawannakul, P., 2017. Chemical profiles and antimicrobial activities of Thai propolis collected from *Apis mellifera*. Chiang Mai J. Sci. 44 (2), 438–444.
Santos, F.A., Bastos, E.M.A., Rodrigues, P.H., de Uzeda, M., de Carvalho, M.A.R., de Macedo Farias, L., Moreira, E.S.A., 2002a. Susceptibility of *Prevotella intermedia/Prevotella nigrescens* and *Porphyromonas gingivalis* to propolis bee glue and other antimicrobial agents. Anaerobe 8 (1), 9–15.
Santos, F.A., Bastos, E.M.A., Uzeda, M., Carvalho, M.A.R., Farias, L.M., Moreira, E.S.A.,

Braga, F.C., 2002b. Antibacterial activity of Brazilian propolis and fractions against oral anaerobic bacteria. J. Ethnopharmacol. 80 (1), 1–7.
Sârbu, C., Moţ, A.C., 2011. Ecosystem discrimination and fingerprinting of Romanian propolis by hierarchical fuzzy clustering and image analysis of TLC patterns. Talanta 85 (2), 1112–1117.
Savka, M.A., Dailey, L., Popova, M., Mihaylova, R., Merritt, B., Masek, M., Le, P., Mat Nor, S.R., Ahmad, M., Hudson, A.O., Bankova, V., 2015. Chemical composition and disruption of quorum sensing signaling in geographically diverse United States propolis. Evid. Compl. Alter. Med. 2015, 472593 10 pages.
Sawaya, A.C., Abdelnur, P.V., Eberlin, M.N., Kumazawa, S., Ahn, M.R., Bang, K.S., Nagaraja, N., Bankova, V.S., Afrouzan, H., 2010. Fingerprinting of propolis by easy ambient sonic-spray ionization mass spectrometry. Talanta 81 (1–2), 100–108.
Seidel, V., Peyfoon, E., Watson, D.G., Fearnley, J., 2008. Comparative study of the antibacterial activity of propolis from different geographical and climatic zones. Phytother. Res. 22 (9), 1256–1263.
Sforcin, J.M., 2016. Biological properties and therapeutic applications of propolis. Phytother. Res. 30 (6), 894–905.
Shimomura, K., Inui, S., Sugiyama, Y., Kurosawa, M., Nakamura, J., Choi, S.J., Ahn, M.R., Kumazawa, S., 2012. Identification of the plant origin of propolis from Jeju Island, Korea, by observation of honeybee behavior and phytochemical analysis. Biosci. Biotech. Biochem. 76 (11), 2135–2138.
Shrestha, S.P., Narukawa, Y., Takeda, T., 2007. Chemical constituents of Nepalese propolis: isolation of new dalbergiones and related compounds. J. Nat. Med. 61 (1), 73–76.
Shuaib, M., Ali, A., Ali, M., Panda, B.P., Ahmad, M.I., 2013. Antibacterial activity of resin rich plant extracts. J. Pharm. Bioallied Sci. 5 (4), 265–269.
Siheri, W., Zhang, T., Ebiloma, G.U., Biddau, M., Woods, N., Hussain, M.Y., Clements, C.J., Fearnley, J.U., Edrada Ebel, R., Paget, T., Muller, S., Carter, K.C., Ferro, V.A., De Koning, H.P., Watson, D.G., 2016. Chemical and antimicrobial profiling of propolis from different regions within Libya. PLoS One 11 (5) e0155355.
Silva, B.B., Rosalen, P.L., Cury, J.A., Ikegaki, M., Souza, V.C., Esteves, A., Alencar, S.M., 2008. Chemical composition and botanical origin of red propolis, a new type of Brazilian propolis. Evid. Compl. Alter. Med. 5 (3), 313–316.
Silva, C.R.B.D., Putarov, T., Orsi, R.D.O., 2013. Pollen spectrum of propolis samples from São Paulo State, Brazil. Acta Sci. Animal Sci. 35 (3), 297–300.
Simone, M., Evans, J.D., Spivak, M., 2009. Resin collection and social immunity in honey bees. Evolution 63 (11), 3016–3022.
Simone-Finstrom, M., Borba, R.S., Wilson, M., Spivak, M., 2017. Propolis counteracts some threats to honey bee health. Insects 8 (2), 46. https://doi.org/10.3390/insects8020046.
Simone-Finstrom, M., Gardner, J., Spivak, M., 2010. Tactile learning in resin foraging honeybees. Behav. Ecol. Sociobiol. 64 (10), 1609–1617.
Simone-Finstrom, M., Spivak, M., 2010. Propolis and bee health: the natural history and significance of resin use by honey bees. Apidologie 41 (3), 295–311.
Simone-Finstrom, M., Spivak, M., 2012. Increased resin collection after parasite challenge: a case of self-medication in honey bees? PLoS One 7 (3), e34601.
Smith, B.H., Cobey, S., 1994. The olfactory memory of the honeybee *Apis mellifer*a. II. Blocking between odorants in binary mixtures. J. Exp. Biol. 195, 91–108.
Solorzano, E.R., Bortolini, C., Bogialli, S., Di Gangi, I.M., Favaro, G., Maldonado, L., astore, P., 2017. Use of a LC-DAD-QTOF system for the characterization of the phenolic profile of the argentinean plant *Zuccagnia punctata* and of the related propolis: new biomarkers. J. Funct. Foods 33, 425–435.
Stevenson, P.C., Nicolson, S.W., Wright, G.A., 2017. Plant secondary metabolites in nectar: impacts on pollinators and ecological functions. Funct. Ecol. 31 (1), 65–75.
Suleman, T., Vuuren, S., Sandasi, M., Viljoen, A.M., 2015. Antimicrobial activity and chemometric modelling of South African propolis. J. Appl. Microbiol. 119 (4), 981–990.
Sumner, L.W., Mendes, P., Dixon, R.A., 2003. Plant metabolomics:large-scale phytochemistry in the functional genomics era. Phytochemistry 62, 817–836.
Tang, T.X., Guo, W.Y., Xu, Y., Zhang, S.M., Xu, X.J., Wang, D.M., Zhao, Z.M., Zhu, L.P., Yang, D.P., 2014. Thin-layer chromatographic identification of Chinese propolis using chemometric fingerprinting. Phytochem. Anal. 25 (3), 266–272.
Teixeira, É.W., Negri, G., Meira, R.M., Salatino, A., 2005. Plant origin of green propolis: bee behavior, plant anatomy and chemistry. Evid. Compl. Alter. Med. 2 (1), 85–92.
Temizer, I.K., Güder, A., Celemli, Ö.G., 2017. Botanic origin and antioxidant activities of a propolis from Irano-Turanian region as nutritional supplements. Istanbul J. Pharm. 47 (3) 2-2.
Tomás-Barberán, F.A., García-Viguera, C., Vit-Olivier, P., Ferreres, F., Tomás-Lorente, F., 1993. Phytochemical evidence for the botanical origin of tropical propolis from Venezuela. Phytochemistry 34 (1), 91–196.
Tomazzoli, M.M., Neto, R.D.P., Moresco, R., Westphal, L., Zeggio, A.R., Specht, L., Costa, C., Rocha, M., Maraschin, M., 2015. Discrimination of Brazilian propolis according to the seasoning using chemometrics and machine learning based on UV-Vis scanning data. J. Integr. Bioinform. 12 (4), 15–26.
Tran, V.H., Duke, R.K., Abu-Mellal, A., Duke, C.C., 2012. Propolis with high flavonoid content collected by honey bees from *Acacia paradoxa*. Phytochemistry 81, 126–132.
Trusheva, B., Popova, M., Koendhori, E.B., Tsvetkova, I., Naydenski, C., Bankova, V., 2011. Indonesian propolis: chemical composition, biological activity and botanical origin. Nat. Prod. Res. 25 (6), 606–613.
Trusheva, B., Stancheva, K., Gajbhiye, N., Dimitrova, R., Popova, M., Saraf, R., Bankova, V., 2016. Two new prenylated stilbenes with an irregular sesquiterpenyl side chain from propolis from Fiji Islands. Record Nat. Prod. 10 (4), 465–471.
Trusheva, B., Todorov, I., Ninova, M., Najdenski, H., Daneshmand, A., Bankova, V., 2010. Antibacterial mono-and sesquiterpene esters of benzoic acids from Iranian propolis. Chem. Cent. J. 4 (1), 8. https://doi.org/10.1186/1752-153X-4-8.
Vargas-Sánchez, R.D., Peñalba-Garmendia, M.C., Sánchez-Escalante, J.J., Torrescano-Urrutia, G.R., Sánchez-Escalante, A., 2016. Pollen profile of propolis produced on the eastern edge of the Sonoran Desert in central Sonora, Mexico. Acta Bot. Mex. 114, 69–86.
Wang, X., Hu, H., Luo, Z., Liu, Y., Zhang, H., 2018. A plant origin of Chinese propolis: *Populus canadensis* Moench. J. Apicult. Res. 57 (2), 228–245.
Wester, P., Lunau, K., 2017. Plant–pollinator communication. Adv. Bot. Res. 82, 225–257.
Wilson, M.B., Brinkman, D., Spivak, M., Gardner, G., Cohen, J.D., 2015. Regional variation in composition and antimicrobial activity of US propolis against *Paenibacillus larvae* and *Ascosphaera apis*. J. Invertebr. Pathol. 124, 44–50.
Wilson, M.B., Spivak, M., Hegeman, A.D., Rendahl, A., Cohen, J.D., 2013. Metabolomics reveals the origins of antimicrobial plant resins collected by honey bees. PLoS One 8 (10), e77512.
Winston, M. L., 1987. The Biology of Honey Bees. Harvard University Press Cambridge MA.
Wolfender, J.L., Rudaz, S., Hae Choi, Y., Kyong Kim, H., 2013. Plant metabolomics: from holistic data to relevant biomarkers. Curr. Med. Chem. 20 (8), 1056–1090.
Wollenweber, E., Asakawa, Y., Schillo, D., Lehmann, U., Weigel, H., 1987. A novel caffeic acid derivative and other constituents of *Populus* bud excretion and propolis bee-glue. Z. Naturforsch. C Biosci. 42 (9–10), 1030–1034.
Wollenweber, E., Buchmann, S.L., 1997. Feral honey bees in the Sonoran Desert: propolis sources other than poplars (*Populus* spp.). Z. Naturforsch. C Biosci. 52 (7–8), 530–535.
Wu, Y.W., Sun, S.Q., Zhao, J., Li, Y., Zhou, Q., 2008. Rapid discrimination of extracts of Chinese propolis and poplar buds by FT-IR and 2D IR correlation spectroscopy. J. Mol. Struct. 883, 48–54.
Xu, L., Yan, S.M., Cai, C.B., Yu, X.P., 2013. Untargeted detection and quantitative analysis of poplar balata PB in Chinese propolis by FT-NIR spectroscopy and chemometrics. Food Chem. 141 (4), 4132–4137.
Yan, S., Zhang, H.-c., Dong, J., 2012. Difference in volatile compounds of propolis from different plant sources. Food Sci 10, 057.
Yang, J., Chen, L.-z., Xue, X.-f., Wu, L.-m., Li, Y., Zhao, J., Wu, Z.-b., Zhang, Y.-n., 2016. A feasibility study on the discrimination of the propolis varieties based on near infrared spectroscopy. Spectrosc. Spectr. Anal. 36 (6), 1717–1720.
Zabaiou, N., Fouache, A., Trousson, A., Baron, S., Zellagui, A., Lahouel, M., Lobaccaro, J.M.A., 2017. Biological properties of propolis extracts: Something new from an ancient product. Chem. Phys. Lipids 207, 214–222.
Zhang, C.P., Zheng, H.Q., Hu, F.L., 2011a. Extraction, partial characterization, and storage stability of β-glucosidase from propolis. J. Food Sci. 76 (1), C75–C79.
Zhang, C.P., Zheng, H.Q., Liu, G., Hu, F.L., 2011b. Development and validation of HPLC method for determination of salicin in poplar buds: application for screening of counterfeit propolis. Food Chem. 127 (1), 345–350.
Zhang, C., Hu, F., 2011. HPLC fingerprints in distinguishing propolis and poplar tree gum. J. Chin. Inst. Food Sci. Technol. 1, 055.
Zhang, T., Omar, R., Siheri, W., Al Mutairi, S., Clements, C., Fearnley, J., Edrada-Ebel, R., Watson, D., 2014. Chromatographic analysis with different detectors in the chemical characterisation and dereplication of African propolis. Talanta 120, 181–190.
Zhou, J., Li, Y., Zhao, J., Xue, X., Wu, L., Chen, F., 2008. Geographical traceability of propolis by high-performance liquid-chromatography fingerprints. Food Chem. 108 (2), 749–759.



Vassya Bankova obtained her PhD in Natural Product Chemistry at the Bulgarian Academy of Sciences (Sofia). She has worked as a guest-researcher at the Ruhr University (Bochum), the University of Saarland (Saarbruecken), the Bandeirante University (Sao Paulo), and the Institute of Molecules of Biological Interest (Naples). In 2004 she became full professor at the Institute of Organic Chemistry with Centre of Phytochemistry, where she is now heading the lab of “Chemistry of Natural Substances”. Her research interests are directed towards the search for new bioactive compounds from unexplored biological subjects (*i.e.*, endemic plants, mushrooms, bee products, just to name a few), development of modern reliable methods of qualitative and quantitative analysis of the active components in complex extracts of natural products. Her special interest is devoted to the bee product propolis (bee glue): its chemistry, plant origin, biological activity, health-promoting potential, analysis and standardization. Prof. Bankova is a member of the International Honey Commission and a part-time lecturer at the University of Sofia. In 2015 she was elected to a corresponding member of the Bulgarian Academy of Sciences.



Milena Popova obtained her Master's degree in chemistry and physics at Sofia University "Sv. Kliment Oxridski". She completed her PhD in natural product chemistry at Bulgarian Academy of Sciences (BAS) in 2004, under the supervision of Prof. Dr. Vassya Bankova. Milena Popova was a postdoctoral researcher at University of Athens, Department of Pharmacy, Division of Pharmacognosy and Chemistry of Natural Product (2007–2008). In 2014 she became Associate Professor at the Institute of Organic chemistry with Centre of Phytochemistry.

Her research interests include chemical profiling, isolation and structural elucidation of bioactive compounds of different products (bee product-propolis, medicinal and endemic plants, mushrooms). Her specific research topic is related to propolis (bee glue) and she is co-author of over 60 papers (incl. book chapters and reviews) on propolis chemistry, botanical origin, biological activity, quality control and standardization. At present Dr. Milena Popova is a President of Bulgarian Phytochemical Society.



Dr. Boryana Trusheva obtained her M.Sc. in organic chemistry from Sofia University (Sofia, Bulgaria) in 2002. She has completed her Ph.D in natural product chemistry at Laboratory Chemistry of Natural Products, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences. She is currently an assistant professor at the same institute. Dr. Trusheva has worked and published in the areas of isolation and structure elucidation of bioactive secondary metabolites from propolis and its processing methods. The current focus of her research is chemical profiling, therapeutic properties and pharmaceutical application of different propolis types.