BOTANICAL ORIGIN AND SEASONAL PRODUCTION OF PROPOLIS IN HIVES OF CENTRAL CHILE

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Abstract – (Botanical origin and seasonal production of propolis in hives of Central Chile). A year round sampling for propolis components at the María Pinto region in Central Chile was assayed. We show here the features of Chilean propolis, using micromorphological assay of pollen grains. Major plant sources included *Eucalyptus globulus*, *Populus alba*, and the native species *Baccharis linearis*, *Peumus boldus*, *Escallonia illinita* and *Quillaja saponaria*. The maximum yield of crude propolis is reached on August and September. The pharmaceutical significance of these findings is discussed.

Resumo - (Origem botânica e produtividade sazonal de própolis na região central do Chile). A observação ao longo de um ano das características botânicas de amostras de uma colméia de María Pinto (região central do Chile) revela que as espécies introduzidas mais utilizadas na produção de própolis são *Eucalyptus globulus* e *Populus alba*, enquanto as nativas são *Baccharis linearis*, *Peumus boldus*, *Escallonia illinita* e *Quillaja saponaria*. A produção de própolis bruta apresenta rendimento máximo nos meses de agosto e setembro. Discute-se a importância farmacêutica desses achados.

Kew words: Propolis, Central Chile productivity, *Baccharis*, *Peumus boldus*, *Escallonia*, *Quillaja*.

Introduction

Propolis is a resinous substance found in *Apis mellifera* hives, and has versatile biological activities (Ghisalberti 1979), including antimicrobial ones, especially against Gram-positive bacteria (Vanhaelen and Valhaelen-Fastre 1979, Focht *et al.* 1993, Steinberg *et al.* 1996, Bretz *et al.* 1998, Montenegro *et al.* 1999). Phenolic components are the main constituents of propolis, and some of those such as pinocembrine (5,7 dihydroxyflavanone), galangine, and cafeic acid and its esters (Kujumgiev *et al.* 1993, Park *et al.* 1998) have antibiotic properties.The botanical origin of propolis found in hives located in zones of the Central Chile sclerophyllous matorral has been diagnosed through the analysis of pollen grains present in the sediment (Valcic *et al.* 1999). Results show that the pollen grains appearing in frequently significant manner in the sample belong to the native species *Baccharis linearis* (Ruiz & Pav.) Person, *Buddleja globosa* Hope, *Peumus boldus* Molina and *Salix humboldtiana* Willd. and to the introduced species *Eucalyptus globulus* Labill. and *Ricinus communis* L. Recent work (Montenegro *et al.* 2000) shows the presence of pollen grains of other species, such as *Aristotelia chilensis* Stuntz, *Cestrum parqui* L'Hér., *Citrus limon* (L.) Burm. f., *Colliguaja odorifera* Molina, *Cryptocarya alba* (Molina) Looser, *Escallonia illinita*, *Eupatorium glechonophyllum* Less., *E. salvia* Colla, *Hypochaeris radicata* L., *Lithraea caustica* (Molina) Hook. & Arn., *Maytenus boaria* Molina, *Populus alba* L., *Quillaja saponaria* Molina and *Trevoa trinervis* in Chilean propolis.

The botanical origin of Chilean propolis has also been traced through the identification of leaf fragments and other vegetative structures of plants present in the sediment, through indicators such as stomata, epidermis and trichomes (Valcic *et al.* 1999). Leaf fragments of *L. caustica*, *T. trinervis*, *Kageneckia oblonga* and *Baccharis linearis* were easily recognized in the propolis samples, due to their characteristic filamentous and glandular trichomes.

Given the importance of native species in the origin of propolis in the Central Zone of Chile, it is necessary to undertake comparative studies with other similar zones, so as to establish an information network. In the same manner, the studies of propolis production through a latitudinal transect can provide information about the variability of quality according to geographical origin. Also, the analysis of source plants compared with the propolis obtained from the same sites can provide additional information useful for the standardization of this resource. Diagnosis of the species responsible for propolis production can lead to the knowledge of native plants showing potential for drug production.

Materials and Methods

Propolis was collected from five hives at María Pinto, Metropolitan Region (33° 31' S, 71° 8' W), 500 m. a. s. l., in Mediterranean type climate region, southwest of Santiago.

Micromorphological analysis was done using a Nikon optical microscope and a scanning electronic microscope (SEM) JEOL JSM 25 SII. Samples for SEM were dried in acetone via CO_2 in a Polaron 33,000 critical point drying apparatus. To analyze pollen grains in the propolis samples, the methane insoluble part of propolis (sediment) was processed by the Erdtmann (1954) acetolysis method. The

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following palynological characteristics were determined: size of the grain, number of colpi, and exine architecture. Comparisons were carried out with pollen samples obtained from plants around the hives. Representative samples are kept in the pollen grain catalog of the botanical laboratory of the Pontifical Catholic University.

To identify the presence of epidermal annexes, such as epidermal glands, trichomes and bud tissues in the propolis samples, thin slides of these were fixed in a mixture of formaldehyde, alcohol and acetic acid 95:5:5 (FAA), embedded in solid paraffin Paraplast ® and stained with safranin and fast green, and were then observed under an optical microscope. For SEM observation, samples were fixed in the same FAA mixture, dehydrated with an acetone series of growing concentrations and dried from pure acetone via CO_2.

Yield was estimated as the weight of samples collected monthly in 1998.

Results

Concerning the palynological origin of propolis, Table 1 shows the total percentages of pollen found in the five samples collected monthly. Results show fourteen pollen types, (χ^2 test, <0.05) and the species appearing in more significant occurrence are: *Eucalyptus globulus*, *Salix humboldtiana*, *Populus alba* and *Baccharis linearis*. The other represented between 2 and 5%. Therefore, the species of the Myrtaceae and Salicaceae families are the most interesting representatives for honeybees when foraging for resins and essential oils for the production of propolis.

The presence of other micromorphologic indicators such as epidermal annexes has a diagnostic value for the identification of the plant sources of propolis. Table 2 shows the species of which there were morphological remains in propolis, not only during the whole year, but especially during the period when plant sources visited by the bees are not in flower.

The production of propolis determined in five hives of María Pinto, Los Canelos property, is shown in Table 3. Maximum amounts can clearly be observed during the months of August and September, 1998 (54-55 %).

Flowering phenology and vegetative stages during the period of study are shown in Table 4. Comparing with results of Table 1, it can be observed that there is a correlation between the presence of pollen in the propolis samples and the flowering phenophase.

The introduced species *E. globulus* has always shown significant values of pollen present in propolis. *Baccharis linearis* has been identified as a source of the propolis in the study area, through morphologic structures only (Table 1).

Table 1. Pollen percent in propolis samples from María Pinto region.

Species	Months											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Aristotelia chilensis	8.0	11.0	0	0	0	0	0	0	0	0	0	0
Baccharis linearis	18.0	21.0	48.0	0	0	9.6	0	0	0	0	0	0
Buddleja globosa	5.7	0	0	0	0	0	0	0	0	0	4.3	7.1
Cestrum parqui	3.3	10.0	14.5	0	0	0	0	0	0	0	0	0
Colliguaja odorifera	0	0	0	0	0	0	8.3	8.7	5.8	10.3	0	0
Cryptocarya alba	2.5	1.2	0	0	0	0	0	0	0	0	0	13.0
Escallonia illinita	4.1	9.3	29.5	0	0	0	0	0	0	0	0	0
Eucalyptus globulus	0	0	0	0	0	87.0	56.0	58.2	57.0	21.1	0	0
Lithraea caustica	9.8	0	0	0	0	0	0	0	0	0	0	14.0
Maytenus boaria	0	0	0	0	0	0	0	0	7.2	14.9	12.0	0
Peumus boldus	0	0	0	0	0	3.3	5.5	12.0	10.0	20.8	0	0
Populus alba	0	0	0	0	0	0	29.0	21.0	19.0	32.8	11.7	0
Quillaja saponaria	13.1	13.0	0	0	0	0	0	0	0	0	0	12.0
Salix humboldtiana	35.2	34.0	7.2	0	0	0	0	0	0	0	71.0	54.0
Total percentage	99.7	100.0	99.2	0	0	100.0	99.0	99.9	100.0	100.0	100.0	100.0

Table 2. Presence of non pollen diagnostic elements, i.e. trichomes or remains of epidermal structures, in propolis samples from María Pinto region.

Species	Months											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Aristotelia chilensis	+		+	+	+	+				+	+	
Baccharis linearis			+	+								
Buddleja globosa	+	+										
Cestrum parqui										+	+	
Colliguaja odorifera					+	+	+				+	+
Cryptocarya alba				+	+							
Escallonia illinita	+		+	+	+					+	+	
Lithraea caustica		+	+	+								
Maytenus boaria												
Peumus boldus	+				+				+	+	+	
Populus alba				+	+	+		+	+			
Quillaja saponaria	+	+	+	+	+			+			+	
Salix humboldtiana	+	+	+									

Table 3. Production of crude propolis in María Pinto region.

Species	Months												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Hive 1	5.6	5.3	4.2	1.3	1.1	2.4	3.6	12.1	8.6	9.3	4.2	6.1	63.8
Hive 2	4.6	4.1	3.6	3.3	2.4	2.1	2.8	8.6	9.2	8.4	10.1	8.6	67.8
Hive 3	4.8	4.3	4.2	3.2	6.3	8.4	8.6	13.6	14.8	9.3	8.4	6.3	92.2
Hive 4	4.6	2.1	2.1	2.3	2.4	6.4	7.3	11.7	11.7	7.4	6.8	6.3	71.1
Hive 5	3.2	3.1	3.6	2.1	2.6	6.3	8.4	8.6	9.3	9.5	9.4	9.2	75.3
% Month	22.8	18.9	17.7	12.2	14.8	25.6	30.7	54.6	53.6	43.9	38.9	36.5	

Table 4. Phenology of species sampled in the vegetation of María Pinto region.

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Aristotelia chilensis								v	v/f	v/f	f	f
Baccharis linearis	f	f						v	v	v	v	v/f
Buddleja globosa									v	v	v/f	v/f
Cestrum parqui	f	f						v	v/f	v/f	v/f	v/f
Colliguaja odorifera								v	v	v	v	v
Cryptocarya alba	v/f									v	v	v/f
Escallonia illinita	f	f					v	v	v	v	v	v/f
Eucalyptus globulus		v	v/f	v/f	f	f						
Lythraea caustica								v	v/f	v/f	v/f	v/f
Maytenus boaria								v/f	v/f	v	v	
Peumus boldus						f	v/f	v	v	v	v	v
Populus alba					v	v	v/f	v/f	v/f	f		
Quillaja saponaria	f							v	v	v	v	v/f
Salix humboldtiana						v	v	v	v/f	f	f	f

v = vegetative f = flower/fruiting

Discussion

The results can pinpoint native species that may have potentiality as drug sources. Therefore, it seems worthwhile to analyze the available information about the species diagnosed as responsible for the origin of propolis, as regards their chemistry and pharmacology.

Aristotelia chilensis (Molina) Stuntz, the maqui, has been fully studied by several groups of phytochemists, who have isolated hydrocarbons, anthraquinone, triterpenes, anthocyanins and alkaloids (Torres and Comin 1975, Bakhuni *et al.* 1976a, Cespedes *et al.* 1990, Bittner *et al.* 1975, He *et al.* 1996b). Some of these products have anticancer and antimicrobial activities, as was demonstrated by the work of Bakhuni *et al.* (1976a, b). This species is widely distributed between the 4th and 10th regions of the country, and is a weed in Juan Fernández, Más a Tierra and Más Afuera (Matthei 1995).

Baccharis linearis (Ruiz & Pav.) Person, an evergreen dioecious shrub, is very abundant in the central zone, a dominant element which is not much attacked by defoliators. However, it has a relationship with the Tephritidae *Rachiptera limbata*, which forms galls in terminal floral buds (Aljaro *et al.* 1984). According to Matthei (1995) it has become a weed from the 3rd to the 10th regions. It has been studied phytochemically for the presence of flavonoids (kaempferol-3-methyl ether, quercetin-3-methyl ether and quercetin-3,3'-methyl ether), Baccharis oxyde, lachnophyllum ether, oleanolic acid, one cromene, two phenylpropanoid derivatives and one ferulic acid ether (Labbe *et al.* 1986, Faini *et al.* 1991, Brown 1994). He *et al.* (1996a) have recently informed about 3 new neoclerodans and a perhydroazulene.

Buddleja globosa Hope, the panguil, is a shrub of the central southern zone of the country. Its chemistry has been described by López *et al.* (1979) and Marín *et al.* (1979), who determined triterpenes and flavonoids, respectively.

Cestrum parqui L'Hér., the palqui, a shrub which is toxic to livestock, contains solasodine (Silva *et al.* 1962). The poisoning of livestock by *C. parqui* is characterized by ataxia, depression, reclination, convulsions and death, mainly due to hepatotoxicity (McLennan & Kelly 1984).

Colliguaja odorifera Molina is a sclerophyllic shrub, typical of the Central Chile sclerophyllous matorral. Two gall-forming insects parasitize *C. odorifera*: *Exurus colliguayae*, which attacks aments, and *Torymus laetus* which does the same with the vegetative buds (Martínez *et al.* 1992). Montenegro *et al.* (1980) determined the phenolic and catechinic tannins and their relationship with palatability to phytophagous insects.

Cryptocarya alba (Molina) Looser, the peumo, is a member of the sclerophyllous forest and has been often diagnosed as a source for Chilean propolis (data not published). Urzúa *et al.* (1975) isolated alkaloids of the benzylisoquinoline type. Montes *et al.* (1988) analyzed the essential oil. Timmermann *et al.* (1995) isolated 10 flavonoids and chlorogenic acid. However, these do not seem to be candidates as antibiotic agents, since they are derived from quercetin, kaempferol or isorhamnetin.

Escallonia pulverulenta Presl. is an iridoid containing shrub. The presence of acacetin-7-methyl ether, kaempferol, rutin and chlorogenic acid has been investigated by Tomassini *et al.* (1993). These authors have isolated iridoids of the dafiloside and geniposide series from this and another species, *E. myrtoidea*. Pinocembrin has been found in *E. illinita* and other species of the same genus (García *et al.* 1990).

Eucalyptus globulus Labill. is the second monoculture of the country and thus could show potential for the production of standardized propolis. Montes *et al.* (1988) carried out the first studies tending to quality control of the species. Erazo *et al.* (1990) examined the twelve *Eucalyptus* species in Central Chile, and found that *E. globulus* produced cineol at a rate lower than that reported in the literature. A substance isolated recently, eucalyptone, has potential as a cariostatic agent (Osawa *et al.* 1996).

Lithraea caustica (Molina) Hook. & Arn., the litre, a tree known because it causes contact dermatitis, as it has components similar to the uroshiols of *Rhus toxicodendron*. Gambaro *et al.* (1986) isolated 3-(pentadec-10-enil) catechol as the allergenic compound.

Maytenus boaria Molina is the maitén. Although there is little data on clinical or pharmacological use, it was recognized early on as a potential source of anticancer drugs by Bakhuni *et al.* (1976a), who identified activity in the lymphocytic P-388 leukemia test model.

Quillaja saponaria Molina, the Panama bark or quillay, is a pharmaceutical and industrial resource which has recently been patented in several parts of the world, including Chile, the United States and Japan (Lagos 1991, Kensil *et al.* 1988, Sugiura *et al.* 1991). Oakenfull (1981) has reviewed its use in industry, including as a foam producing agent for soft drinks and confectionery products. The physicochemical parameters of the process of cholesterol separation in butteroils are being studied (Sundfeld *et al.* 1993a, b). Derivatives of the quillaic acid have been reported recently by Guo *et al.* (1998): 3-O-beta-D-galactopyranosyl-(1→2)-beta-D-glucopyranosiduronic acid, 3-O-alpha-L-rhamnopyranosyl-(1→3)-[beta-D-galactopyranosyl-(1→2)]-beta-D-glucopyranosiduronic acid and 3-O-beta-D-xylopiranosyl-(1→3)-[beta-D-galactopyranosyl-(1→2)]-beta-D-glucopyranosiduronic acid, respectively. The 4-methyl-7-hydroxyphthalideglycoside was isolated from the methanol extract of the bark of *Q. saponaria*, and the structure corresponds to 7-O(-)[beta-glucopyranosyl-(1→6)-beta-arabinopyranosyl]-7-hydroxy-4-methyl-1[3H]-isobenzofuranone (Steinbeck *et al.* 1995). A substance adjuvant for vaccines against the foot and mouth diseases was isolated by Dalsgaard (1974). Reccia *et al.* (1995) studied the increase in permeability to aminoglucoside antibiotics. Chao *et al.* (1998) in a similar manner on semisynthetic products studied the effects of improved transport of drugs, such as insulin and aminoglucoside antibiotics.

Peumus boldus Molina is a tree that grows down to the Osorno latitude. It has been studied and incorporated into several pharmacopoeias (FCh III, HAB1) and is an important export resource. During the last decade it represented 3%-5 % of the exports of the sector. A review about the *P. boldus* chemistry has been presented by Speisky and Cassels (1994). The presence of 1% to 2% of essential oils in the boldo leaf, with *p*-cimol, cineol, ascaridol among other components, and their seasonal variations have been reported by Montes *et al.* (1980) and Steinnegger and Hänsel (1988). Gottland *et al.* (1995) studied the effect of a dry boldo extract on the oro-caecal transit in healthy volunteers, which takes longer than with a placebo. The anti-inflammatory and antipyretic properties were determined by Backhouse *et al.* (1994).

Populus alba L. var. *italica*, the poplar, is an introduction from Europe, where it is widely used by bees for the production of propolis. *Populus alba* belongs to the section *Leuce*, subsect. *Albidae*, close to *P. grandidentata* and *P. tomentosa*. These species contain hydrocarbons in the bud exudates only, as was observed in the analysis of the main components (Greenaway *et al.* 1991). But some populations contain pinocembrin, which is usual in other sections of the genus.

Salix humboldtiana Willd. (*S. chilensis* Molina), the Chilean willow, is a tree that grows in humid areas between Copiapó and Concepción. It contains 0,88 % salicilin. Methanol extracts would have a cytotoxic activity against CA 9 KB and P38% leukemia, besides an ADN binding effect (Pezzutto *et al.* 1991).

The species whose pollens are more frequent in the María Pinto propolis have potential as pharmaceuticals and as probes for the detection of microbiologically active components. For example, the presence of pinocembrin *in Escallonia pulverulenta*, and probably in *Populus* species. A number of diterpenes have been found in *Baccharis*, some of them bioactive.

The less known species, phytochemically and pharmacologically, are *Colliguaja odorifera* Molina and *Maytenus boaria* Molina. There are insufficient data on *Buddleja globosa*, a plant widely used in phytomedicine, thus deserving deeper studies about its use in biomedical practice.

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