Phenolic Composition of Propolis from China and from South America

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Flavonoids and other phenolic compounds were determined in 15 samples of propolis originating from China, from Brazil, and from Uruguay. A total of 24 compounds were identified using mainly HPLC and a few other analytical methods. The most abundant compounds found were benzoic acid and benzaldehyde derivatives, flavones, flavonols, and flavanones. 80% of the samples contained at least 22 g/100 g of flavonoids, primarily acacetin, isorhamnetin, apigenin, and pinocembrin. The flavonoid patterns were sufficiently distinctive to permit discrimination between propolis from China, from Uruguay, and from Brazil.

Introduction

Propolis is a complex mixture of natural, sticky, gummy and resinous components collected by honey-bees *(Apis mellifera)* from the buds of various trees and used for the asepsis of the hive (Hausen *et al.,* 1987; Nagy *et al.,* 1988; Greenaway *et al.,* 1990a; Bankova *et al.,* 1991; Serra Bonvehí *et al.,* 1994). Bees use propolis to repair the hives, to strengthen and join the cells, and to avoid the entrance of water into the hive, thus creating an unfavorable environment for microorganism development. The honey-bee modifies the original composition of plant resins by extracting resinous substances and mixing them with hypopharyngeal gland secretions, especially β-glycosidases. Flavonoid heterosides are hydrolyzed to free aglycones increasing the pharmacological action of the resulting product (Vanhaelen and Vanhaelen-Fastré, 1979a, b). Poplars (*Populus* spp.), birches (*Betula* ssp.), elms (*Ulmus* spp.), pine trees (*Pinus* spp.), oaks (*Quercus* spp.), willows (*Salix* spp.), chestnut trees (*Aesculus hippocastanum* L.), spruce (*Picea* spp.), and ashes (*Fraxinus* spp.) are among the more important resin sources in northern hemisphere. These origins may account for colour, smell and biological differences of propolis. Phenolic compounds constitute the largest fraction of propolis, consisting mainly of terpenic substances, benzoic acid derivatives, benzaldehyde derivatives and flavonoids (Wollenweber and Dietz, 1981; Bankova *et al.,* 1982; Marekov *et al.,* 1984; Suchy *et al.,* 1985; García-Viguera *et al.,* 1992). Several flavonoids with pharmacological activities (*e.g.* spasmolytic, anti-inflammatory, anti-ulcerous, and bacteriostatic) have been identified in propolis composition. The more important pharmacological flavonoids identified in propolis are flavones, flavonols, flavanones, and dihydroflavonols (Bankova *et al.,* 1982; Greenaway *et al.,* 1987). The therapeutic characteristics of propolis have increased interest in propolis composition. Other components such as phenolic acids and esters, aromatic aldehydes and alcohols, sesquiterpenes also show pharmacologic activity (Bankova *et al.,* 1987; Wollenweber *et al.,* 1987). Taking into consideration all the knowledge gathered on phenolic compounds of propolis, this study is focused on this fraction, identifying the main flavonoids.

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Materials and Methods

Propolis samples

Fifteen samples from different geographic origins and varying presentations (powder and raw) were analyzed (Table I). The origin and plant taxa that contributed to the propolis we analyzed were Anhui province (China) [*Robinia pseudacacia* L., *Populus* spp. (Aigeiros section), *Ulmus* spp., *Morus* spp., *Pyrus* spp., *Prunus* spp., *Salix* spp. and *Melia azederach* L.], from Uruguay (*Eucalyptus globulus* L., *Populus* spp., *Betula* spp. and *Salix* spp.), and from Brazil (*Citrus sinensis* L., *Coffea*

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Table I. Propolis samples.

Sample No.	Geographical origin	Presentation
1	Brazil	natural
2	Uruguay	powder
3	Uruguay	powder
4	Uruguay	powder
5	China	natural
6	China	natural
7	China	natural
8	Uruguay	powder
9	Uruguay	powder
10	Uruguay	powder
11	China	natural
12	China	natural
13	China	natural
14	China	natural
15	China	natural

arabica L., *Saccharum officinarum* L. and *Eucalyptus* spp.). Once in the laboratory, they were kept in darkness and at room temperature.

Instrumentation

A Shimadzu Model UV-160 A double-beam spectrophotometer with 1 cm quartz absorption cells was used for all measurements. HPLC-UV was carried out on a HPLC system consisting of Model 590 Waters Associate LC pumping units, a Model 712 WISP Rheodyne valve loop injector fitted onto a 20 µl loop, and a Waters Associate Model 996 photodiode array detector.

Data processing

Chromatographic data from HPLC and UV were processed on NEC 486/66i computing integrators.

Reagents and standards

Solvents were analytical (Panreac, Barcelona, Spain) and HPLC (Merck, Darmstadt, Germany) grade. Laboratory deionized water was further purified using a vacuum filter (0.45 µm, Schleicher & Schuell, Dassel, Germany). Ferulic and coumaric acids were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Acacetin, apigenin, galangin, kaempferol, quercetin, hesperetin, rutin flavonoids and 4-hydroxybenzoic acid ethyl ester were obtained from Carl Roth GmbH + Co. (Karlsruhe, Germany). Vanillin was from Carlo Erba (Milano, Italy). Caffeic and cinnamic acids, pinocembrin and chrysin flavonoids were from Fluka Chemika (Buchs, Switzerland). Finally, 3,4-dihydroxybenzoic acid, isorhamnetin, sinapic acid, naringin, and tectochrysin were obtained from Extrasynthèse (Genay, France).

Total phenols

The sample (0.50 g) of finely ground and unwaxed propolis was extracted by agitating with 70% methanol (v/v). Phenols in the extract were determined with Folin-Ciocalteu reagent (RFC). A blank was prepared by agitating an aliquot of the extract at pH 3.5 with insoluble polyvinylpolypyrrolidone (PVP). Absorbance was read at 760 nm, and phenols were determined using a calibration curve for 5, 25, 50, 100, 150 and 200 ppm of gallic acid (Marigo, 1973).

Flavonoids

The total flavonoid content was estimated in 0.5 g of finely ground and unwaxed propolis. 1 ml of 0.5% hexamethyltetramine (w/v), 20 ml of acetone, and 2 ml of 0.10 N HCl were added to the sample and set to boil with reflux for 30 min. The resulting solution was filtered and the volume was leveled at 100 ml with acetone, the residue being washed with 20 ml of acetone. 10 ml of the extract were introduced in a separation funnel, along with 20 ml of H_2O and 25 ml of ethyl acetate. Extraction with ethyl acetate was carried out three times. The extract was washed twice, using 50 ml H_2O each time, and diluted to 100 ml with ethyl acetate. The total flavonoid content was determined in 10 ml of the extract using 1 ml of 2% $AlCl_3$ in methanol solution (5% acetic acid in methanol) according to the method described by Lebreton *et al.* (1967). Absorbance was read at 425 nm, and flavonoid percentage was estimated using two calibration curves at 8, 16, 24 and 32 ppm of galangin and rutin.

HPLC analysis of phenolic compounds was performed according to that of Amiot *et al.* (1989). The sample (0.40 g) of finely ground and unwaxed propolis was dissolved in 25 ml of ethyl acetate; then 12.5 ml of 40% $(NH_4)_2SO_4$ and 2.50 ml of 20% HPO_3 were added and the flask was agitated for 20 min. The solution was poured into a separation funnel, the top phase was collected, and the

extraction process was repeated. The organic phases were collected into a 100 ml flask and then concentrated to dryness under reduced pressure. The sample was redissolved in 20 ml of methanol, filtered through 0.45 μm mesh nylon (Lida Manufacture Corporation), and leveled to 25 ml. HPLC was performed following these steps: Nucleosil C_{18} column (10 μm) (4.6 mm i.d. × 250 mm); photodiode array detector at 278–282 nm and 278–350 nm; solvents: a) bidistilled water, pH 2.6 (with H_3PO_4), and b) methanol; flow rate: 2 ml/min; 0% methanol to 100% methanol in 33 min of linear gradient; loop, 20 μl. Phenolic compound quantitation was achieved by the absorbance relative to external standards.

Phenolic compound identification

The different phenolic compounds were identified by their UV spectra which had been recorded with a photodiode array detector coupled to the HPLC, bathochromic movement of band I (320–380 nm) and band II (240–270 nm) using hydroxylation, methylation and metallic complexes in accordance with Markham (1982), as well as co-chromatography with pertinent markers.

Sugar spectrum

Carbohydrates were determined based on the analyses of their oxime trimethylsilyl derivatives by the gas chromatographic method of Serra Bonvehí and Bosch Callís (1989), using a Sigma 2B gas chromatograph and quantified on a Sigma 15 (Perkin-Elmer) microprocessor.

Statistical analysis

Chemical analyses were performed in triplicate. Data obtained from the cluster analysis (Vogt and Nagel, 1992) and measurement were subjected to the analysis of variance, and the least significant difference (lsd) was calculated using SAS (1985).

Results and Discussion

Table II shows the detected chromatographic peaks in elution order, average relative retention time (RRT), absolute retention time (RT), and name attributed to each identified compound.

Table II. Relative (RRT) and absolute (RT) retention times.

Components	RRT	V_{max}	V_{min}	RT	V_{max}	V_{min}
1. Gallic acid	0.28	0.29	0.27	5.73	6.70	5.35
2. 3,4-Dihydroxy-benzoic acid	0.34	0.35	0.33	8.74	9.10	8.46
3. Caffeic acid	0.45	0.46	0.42	10.99	11.36	10.45
4. Vanillin	0.49	0.50	0.46	12.30	12.90	11.66
5. Ferulic acid	0.57	0.58	0.54	14.57	15.40	13.56
6. Sinapic acid	0.60	0.61	0.57	15.25	16.13	14.73
7. *p*-Coumaric acid	0.63	0.64	0.60	15.92	16.93	15.06
8. Naringin	0.66	0.68	0.63	16.48	17.16	15.76
9. Rutin	0.71	0.71	0.69	18.01	19.16	17.50
10. 4-Hydroxybenzoic ethyl ester	0.75	0.77	0.74	19.19	20.43	18.60
11. *o*-Cinnamic acid	0.76	0.79	0.74	19.47	20.86	19.00
12. Quercetin	0.79	0.81	0.77	20.11	21.50	19.56
13. Hesperitin	0.83	0.84	0.81	21.26	22.66	19.43
14. Pinobanksin	0.85	0.87	0.85	21.85	23.53	20.17
15. Kaempferol	0.90	0.90	0.88	22.65	24.40	22.30
16. Apigenin	0.93	0.93	0.92	23.60	25.43	23.36
17. Isorhamnetin	0.94	0.94	0.93	23.87	25.73	23.50
18. Galangin	0.96	0.97	0.95	24.44	26.36	24.00
19. Chrysin	0.98	0.98	0.97	25.00	27.10	24.50
20. Acacetin	1.00	–	–	25.50	27.50	25.00
21. Unknown	1.04	1.05	1.03	26.56	28.00	25.60
22. Pinocembrin	1.07	1.08	1.06	27.20	29.43	26.63
23. Pinostrobin	1.10	1.10	1.08	27.79	30.02	26.92
24. Tectochrysin	1.13	1.14	1.12	28.71	30.20	28.20
25. Unknown	1.16	1.17	1.14	29.31	31.00	28.66
26. Rhamnetin	1.21	1.24	1.20	30.52	32.03	29.50.

The following compounds were identified: i) derivatives of benzoic acid (C_6–C_1), including 3,4-dihydroxybenzoic acid, 4-dihydroxybenzoic ethyl ester (protocatechuic acid) and gallic acid; ii) cinnamic acid derivatives (C_6–C_3), including caffeic, ferulic, sinapic and *p*-coumaric acids;

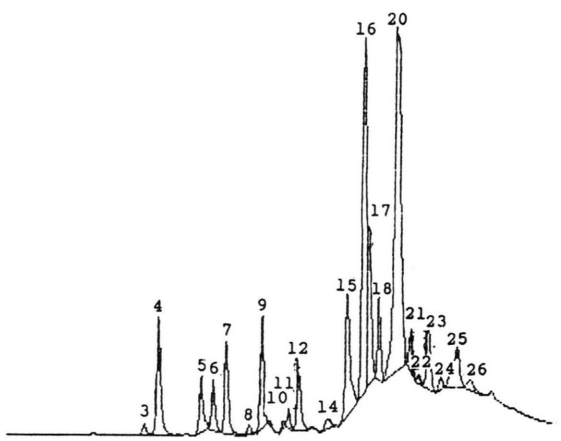

Fig. 1. HPLC phenol profiles of propolis.

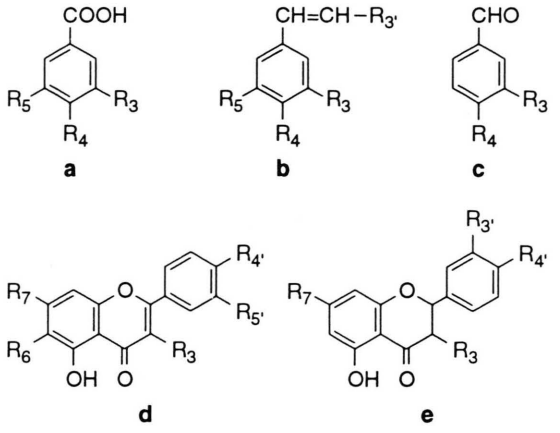

Fig. 2. Phenolic and flavonoid compounds isolated from propolis.

a) Benzoic acid derivatives (C_6-C_1).

	R_3	R_4	R_5
Benzoic acid	H	H	H
3,4-Dihydroxybenzoic acid	OH	OH	H
4-Hydroxybenzoic acid	H	OH	H
Gallic acid	OH	OH	OH

b) Hydroxycinnamic acids (C_6-C_3).

	R_3	R_4	R_5	$R_{3'}$
Caffeic acid	OH	OH	H	COOH
Ferulic acid	OCH_3	OH	H	COOH
Sinapic acid	OCH_3	OH	OCH_3	COOH
p-Coumaric acid	H	OH	H	COOH

c) Benzaldehyde derivatives.

	R_3	R_4
Vanillin	OCH_3	OH

d) Flavonols and flavones ($C_6-C_3-C_6$).

	R_3	R_6	R_7	$R_{4'}$	$R_{5'}$
Rutin	H	H	OH	OH	OH
Quercetin	OH	H	OH	OH	OH
Kaempferol	OH	H	OH	OH	H
Apigenin	H	H	OH	OH	H
Isorhamnetin	OH	OH	OH	OH	OCH_3
Galangin	OH	H	OH	H	H
Chrysin	H	H	OH	H	H
Acacetin	H	H	OH	OCH_3	H
Tectochrysin	H	H	OCH_3	H	H
Rhamnetin	OH	H	OCH_3	OH	OH

e) Flavanones ($C_6-C_3-C_6$).

	R_3	R_7	$R_{3'}$	$R_{4'}$
Hesperitin	H	OH	OH	OCH_3
Naringin	H	H	H	OH
Pinobanksin	OH	OH	H	H
Pinocembrin	H	OH	H	H
Pinostrobin	H	OCH_3	H	H

iii) benzaldehyde derivatives, vanillin; iv) flavonoids ($C_6-C_3-C_6$), including flavones, flavonones, and flavanones (see Fig. 1 and 2). No chlorogenic acid was identified. The identification of the phenolic fraction required: extraction, hydrolysis, aglycone separation and purification. The honeybee segregates β-glucosidase during propolis processing, causing the enzymatic hydrolysis of glycosides to free aglycones. Without chemical hydrolysis the following free sugars can be identified by gas chromatography: glucose, fructose, galactose, arabinose, sucrose and maltose (Table III). Once propolis has been hydrolyzed (Sabatier *et al.*, 1992), no other components are detected and the percentage of identified free sugars was negligible. The HPLC profile of the phenolic compounds present in the propolis samples indicated the presence of 26 components. Of those 26, we were able to identify 24 using the described methods. Some additional extractions were performed to improve analytical methodology. Of the various solvents we used, 70% methylic alcohol for spectrophotometry and ethyl acetate for HPLC have provided the best recuperation ratios. Minimum recuperation has been 53% for *p*-hydroxybenzoic acid and a maximum of 118% for *p*-hydroxybenzaldehyde, with an average of 75% for most components. Reproducibility of the analyses was ± 10%. Concentrations higher than 1 g/100 g of the detected and identified phenolic components were found for: i) the benzoic acid derivatives, vanillin and 4-hydroxybenzoic acid; ii) the hydroxycinnamic acid, ferulic acid; iii) the flavonoids rutin, quercetin, kaempferol, apigenin, isorhamnetin, acacetin, pinocembrin, and tectochrysin. Except for sample No. 2, total phenols ranged between 18.7 and 33.10 g/100 g. 80% of the samples showed a minimum content of not lower than 20 g/100 g (Table IV).

According to the results shown in Table V, the spectrophotometric values had an approximate average variability of 4 g/100 g inferior to the chromatographic values. Within the phenolic fraction, flavonoids were the most abundant, representing more than 80%. Flavonoids were also quantified by spectrophotometry and chromatography, showing an average difference of 18 g/100 g (Table V). In order to ascertain if this difference was caused by the spectrophotometric analyses, quantification was performed using two calibration curves for

Table III. Carbohydrates composition (g/100 g).

Sample No.	Ara	Sugar Fru	Glc	Suc	Mal
6	tr.	0.88	0.69	0.92	0.10
11	tr.	0.62	0.25	2.24	0.20
12	tr.	0.57	0.22	2.16	0.14
13	tr.	0.88	0.68	0.77	0.10
14	tr.	0.73	0.58	1.17	0.13
15	tr.	0.69	0.62	1.45	0.16

Ara, arabinose; Fru, fructose; Glc, glucose; Suc, sucrose; Mal, maltose; tr., traces.

Table IV. Phenols and flavonoids content (g/100 g).

Sample No.	Phenols	Flavonoids	% Fla/Ph
1	18.72/18.70	3.00/18.10	96.70
2	10.10/13.10	3.00/ 9.60	73.10
3	21.70/23.10	4.70/20.50	88.70
4	20.80/22.14	5.30/20.30	91.70
5	19.90/26.70	6.40/25.00	93.50
6	18.80/18.80	5.50/18.30	97.10
7	20.00/22.90	6.60/22.10	96.50
8	23.20/28.90	5.30/25.00	86.60
9	24.40/31.20	5.10/27.00	86.60
10	25.00/29.40	4.10/25.30	86.00
11	24.80/29.60	5.50/25.50	86.30
12	22.20/25.80	5.80/22.20	86.10
13	25.40/28.90	4.60/25.30	87.50
14	26.40/27.60	3.90/23.30	84.40
15	28.60/33.10	5.70/26.60	80.50

Ph, phenols; Fla, flavonoid; spectrophotometric method/ chromatographic method.

Table V. Recovery methods in apigenin evaluation.

Component	Spectrophotometry ppm	recovered [%]	Chromatography ppm	recovered [%]
Apigenin	10	18.70	10	95.70
	100	13.20	100	93.20

Spectrophotometry: calibration referent galangin standards.

galangin and rutin. No significant differences ($p < 0.05$) were found between the two results. The methods were tested for accuracy, evaluating 10 and 100 ppm of apigenin, one of the main components in propolis. According to the results obtained (Table V), spectrophotometry provided low precision when assessing flavonoids. As the concentration of acacetin increased, the accuracy of the method decreased detecting only 13% of the real acacetin percentage. Since these were the main fraction in the composition of propolis, spectrophotometric methods were not reliable as they could only provide approximate values of total flavonoids. Chromatography detected that 80% of the samples contained at least 22 g/100 g of flavonoids, with not less than 8 components. Most samples showed at least 15 identified compounds such as phenols, flavones, flavonols, and flavanones (Table VI). Acacetin and apigenin were the most abundant. Isorhamnetin, pinocembrin, quercetin, rutin and vanillin however also appeared in smaller proportions. The qualitative composition of the 15 samples was surprisingly similar; however, they did show large quantitative differences. Variance analysis showed a significant difference ($p < 0.01$) in total phenol, flavonoid and active component contents. The analysis reported here shows that flavonoids from poplar bud exudates and propolis in the British Isles and continental Europe to be markedly different from that of propolis-derived flavonoids from China and South America (Greenaway *et al.*, 1987, 1988, 1990a, b; Tomás Barberán *et al.*, 1993).

Fig. 3 illustrates the results obtained in carrying out cluster analyses of the propolis using the standardized mean values of the thirteen most diagnostic variables: vanillin, ferulic acid, rutin, 4-hydroxybenzoic ethyl ester, quercetin, kaempferol, apigenin, isorhamnetin, galangin, acacetin, pinocembrin, tectochrysin and total phenol com-

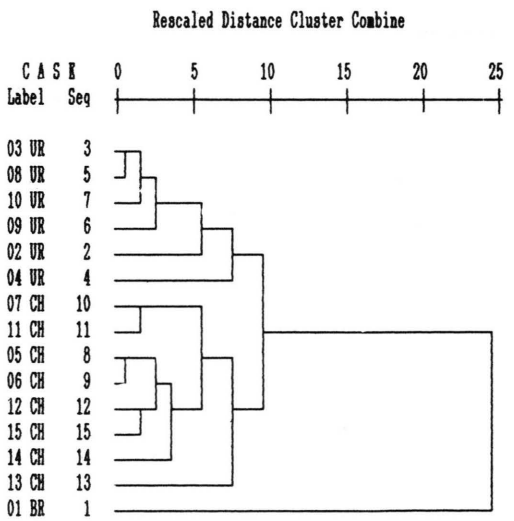

Fig. 3. Dendrogram of average propolis.

Table VI. Phenolic components (HPLC) (g/100g).

Component	Sample No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Gallic acid	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–
3,4-Dihydroxy-benzoic acid	–	–	–	–	–	–	0.10	0.04	–	–	–	0.03	0.03	0.06	–
Caffeic acid	–	–	–	–	0.23	0.29	–	–	–	–	–	–	0.07	0.17	0.21
Vanillin	1.35	0.14	0.06	–	1.36	1.70	1.16	0.29	0.88	–	1.65	1.41	2.37	1.95	2.49
Ferulic acid	0.40	0.40	0.24	0.38	1.16	0.96	1.66	0.16	0.75	0.45	1.00	0.78	0.46	0.99	1.25
Sinapic acid	–	0.06	–	–	0.33	0.31	0.39	–	0.26	0.18	0.22	0.31	0.14	0.36	0.95
p-Coumaric acid	–	0.19	0.10	0.16	0.60	0.78	0.58	0.02	0.38	0.03	0.79	0.58	0.63	0.78	0.97
Naringin	0.29	–	0.16	–	–	–	–	–	–	–	–	–	1.35	–	–
Rutin	0.42	1.55	0.65	1.39	3.18	3.79	3.44	0.48	1.71	1.04	3.70	3.09	3.17	4.09	3.84
4-Hydroxybenzoic ethyl ester	1.48	0.96	0.19	–	0.18	0.46	0.12	0.05	0.23	1.03	0.08	0.19	0.14	0.09	0.73
o-Cinnamic acid	0.01	0.32	0.17	0.25	0.26	0.23	0.11	0.09	0.12	0.15	0.29	0.28	0.12	0.15	0.08
Quercetin	0.87	1.79	1.05	0.94	1.35	1.19	1.43	1.30	1.35	1.33	0.97	0.97	1.10	0.95	1.25
Hesperetin	0.08	0.04	0.22	–	–	0.13	0.21	–	–	–	0.11	0.14	0.12	–	–
Pinobanksin	0.17	0.20	0.17	–	0.21	0.15	0.12	0.16	0.18	0.22	0.16	0.04	0.09	0.10	0.22
Kaempferol	0.05	2.02	1.14	2.13	0.67	0.56	1.28	0.30	0.73	1.38	0.37	0.64	0.28	0.15	0.79
Apigenin	1.04	5.32	3.17	5.86	4.58	5.37	6.70	2.79	3.85	3.47	6.69	3.95	5.74	5.52	4.29
Isorhamnetin	–	2.41	1.81	–	2.62	2.49	2.48	1.91	1.75	1.48	2.63	1.66	–	1.34	1.94
Galangin	0.16	0.33	0.24	0.99	0.73	0.98	0.69	0.25	0.45	0.31	0.70	0.76	1.98	0.86	0.76
Chrysin	0.49	0.07	–	–	0.01	–	–	0.11	0.06	0.12	0.11	0.01	–	0.09	0.02
Acacetin	0.57	6.86	6.66	7.49	8.47	8.71	6.17	7.14	7.35	6.75	6.94	7.29	8.86	6.97	7.58
Not identified	0.88	0.07	0.21	–	0.19	0.39	0.22	0.18	0.10	0.08	0.18	0.28	0.76	0.05	0.14
Pinocembrin	3.46	1.73	0.74	0.94	1.38	1.68	1.37	1.23	1.16	1.66	1.45	1.52	0.90	1.68	1.73
Pinostrobin	–	0.19	–	–	0.26	0.09	0.14	0.09	0.12	0.09	0.07	0.18	0.05	0.08	0.26
Tectochrysin	0.48	1.57	1.41	1.95	0.84	0.36	0.52	1.37	1.08	1.56	1.00	0.87	0.08	0.84	0.69
Not identified	0.27	0.15	0.06	0.06	0.35	0.41	0.13	0.08	0.20	0.10	0.40	0.59	0.30	0.18	0.32
Rhamnetin	0.63	0.41	0.26	0.35	0.19	0.21	–	0.33	0.21	0.42	–	0.12	0.14	–	1.72
Total	13.10	26.70	18.80	22.90	28.90	31.20	29.40	18.70	23.10	22.10	29.60	25.80	28.90	27.60	33.10

Table VII. Correlations between phenolic components.

Correlations	Van	Feru	Rut	Hydr	Quer	Kaem	Apig	Isor	Gal	Acac	Pino	Tect
Van	1.000	0.573	0.790^{+}	−0.080	−0.303	−0.668*	0.259	−0.021	0.607	0.131	0.203	−0.859^{+}
Feru		1.000	0.806^{+}	−0.208	0.122	−0.128	0.531	0.498	0.215	0.192	0.034	−0.494
Rut			1.000	−0.365	−0.073	−0.277	0.696*	0.345	0.605	0.485	−0.128	−0.576
Hydr				1.000	0.178	0.052	−0.552	−0.181	−0.430	−0.646*	0.805^{+}	−0.048
Quer					1.000	0.469	0.152	0.548	−0.232	0.271	−0.147	0.188
Kaem						1.000	0.279	0.054	−0.159	0.175	−0.323	0.687*
Apig							1.000	0.281	0.592	0.592	−0.492	−0.091
Isor								1.000	−0.325	0.354	−0.184	0.030
Gal									1.000	0.052	−0.374	−0.505
Acac										1.000	−0.807^{+}	0.045
Pino											1.000	−0.296
Tect												1.000

Van, vanillin; Feru, ferulic acid; Rut, rutin; Hydr, 4-hydroxybenzoic ethyl ester; Quer, quercetin; Kaem, kampferol; Apig, apigenin; Isor, isorhamnetin; Gal, galangin; Acac, acacetin; Pino, pinocembrin; Tect, tectochrysin; 2-tailed signif.: $^{+}$, 0.001; *, 0.01.

pounds. The structure of the dendrograms and the relative D^2 distance for which the propolis are separated showed the degree to which the single variables are taxonomic, and for which propolis. It was possible to separate different groups between flavonoid patterns and botanical and geographical origins. The flavonoids pattern of propolis we have studied were sufficiently distinctive to permit the discrimination of propolis from China, from Uruguay, and from Brazil. An examination of the principal component of the dendrogram generated by average linkage (between groups) could indicate

the more effective variables in propolis separation. In addition, a statistical correlation study was performed between components. The statistical results for correlation coefficient and significance level are shown in Table VII. Quercetin, isorhamnetin, and galangin were not correlated with any other flavonoid. Between flavonols, only apigenin is positively correlated with rutin, and tectochrysin with kaempferol.

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