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# Some Chemical Composition and Biological Activity of Northern Argentine Propolis

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Twenty-five samples of propolis were collected from seven different regions in northern Argentina; ethanolic extracts of propolis were prepared from all samples, and the respective samples were examined for UV absorption spectra, RPHPTLC, RPHPLC, antimicrobial activity, antiradical activity, and total phenolic content. It was found that 16 of the 25 samples showed a phenolic profile similar to that found in samples from southern Brazil and corresponding to poplar-based propolis and that the rest of the samples showed a different profile and higher antimicrobial and antiradical activities.

**KEYWORDS: Propolis; flavonoids; *Apis mellifera*; poplar resinous exudates; antiradical activity; antimicrobial activity**

## INTRODUCTION

Propolis is a resinous product collected by bees (*Apis mellifera*) from tree exudates, mainly resins of leaf bud mixed with beeswax to form a sealing material in their honeycombs, smooth out the internal walls, and protect the entrance against intruders (*1*). The first report on the use of propolis as a folk medicine dates back to 3000 B.C. (*2*), and recently propolis has also been extensively used in food and beverages to improve health and prevent diseases (*3*−*5*).

The composition of raw propolis varies with the plant source; in general, it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% various other substances, including organic debris (*6*). The analyses of the phenolic compounds present in *Populus nigra* bud exudates clearly support that it is the origin for propolis in continental Europe, North America, western Asia, and New Zealand (*7*−*9*). It is also reported that in areas where poplars are not native plants, such as Australia and equatorial regions in South America, bees that use propolis will seek other plants from which to gather exudates. We have collected 500 samples of propolis obtained by Africanized *Apis mellifera* in Brazil; the respective propolis samples were extracted with ethanol, and those extracts were analyzed by the appearance of measurement of absorption spectra by UV spectrophotometry, reversed phase high-performance thin-layer chromatography (RPHPTLC), and reversed-phase high-performance liquid chromatography (RPHPLC). In accordance with the results of these analyses, the propolis samples were classified into 12 groups (*10*, *11*). It was found that the varieties of propolis are dependent on geographical location because of plant ecology. It was also found that among 12 groups of propolis, the chemical composition of group 3 showed the same profile as poplar resinous exudates in southern Brazil, where poplars were planted by European immigrants (*12*−*14*). We have also observed that bees were visiting mainly a grove of poplar trees to collect resins. Furthermore, we have investigated some propolis samples from Uruguay and Argentina and found that the origin of propolis is resins of the poplar tree. Therefore, the objective of this research was to investigate the chemical composition and some biological activity of propolis in northern Argentina.

## MATERIALS AND METHODS

**Propolis.** Twenty-five samples of propolis were collected in northern Argentina, and one sample represents propolis from one beehive. The ethanolic extract of propolis was prepared as described by Park et al. (*14*). The respective propolis sample (∼30−50 g) was frozen in a freezer and then immediately ground to a fine powder with a Waring blender. Then 2 g of the powder was mixed with 25 mL of 80% ethanol and shaken at 70 °C for 30 min. After extraction, the mixture was centrifuged to give ethanolic extracts of propolis, and the supernatants were used for analysis.

**Measurement of the Absorption Spectra of Ethanolic Extracts of Propolis.** The extracts, 25 μL, were mixed with 30 mL of 80% ethanol, and the mixtures were scanned to obtain the absorption spectra at wavelengths between 200 and 600 nm by UV spectrophotometer.

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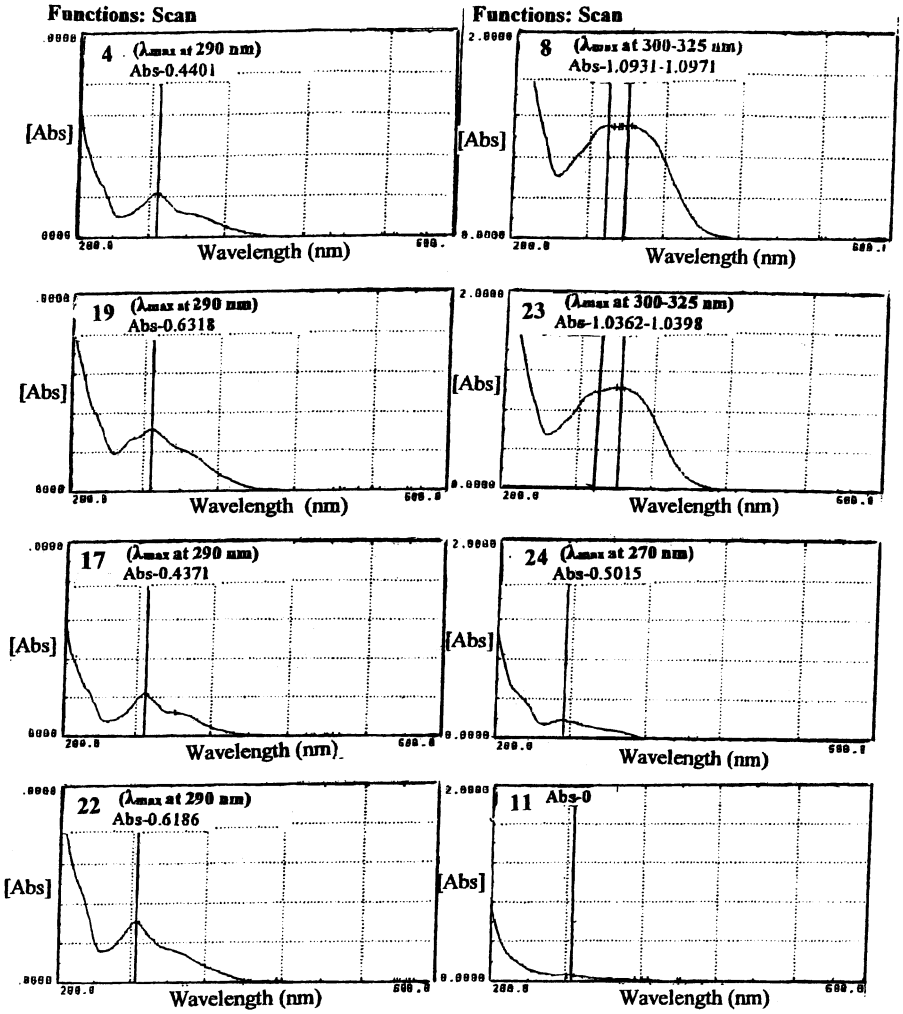



**Figure 1.** Measurement of absorption spectra of ethanolic extracts of propolis.

**RPHPTLC.** Precoated plates of silica gel RP-18 $F_{254}S$ for RPHPTLC were purchased from Merck Co. The method was described by Park et al. (*14*). A 3 $\mu$L portion of the ethanolic extract of propolis was applied to the lower edge of the plate, and ascending chromatography was run using a mobile phase of ethanol/water (55:45, v/v). The detection of flavonoids was carried out using UV visualization at 366 nm.

**RPHPLC.** Analysis of flavonoids from ethanolic extracts of propolis was performed by RPHPLC with a liquid chromatograph equipped with a YMC Pack ODS-A column (RP-18, column size = 4.6 × 250 mm; particle size = 5 $\mu$m) and a photodiode array detector (SPD-M10A, Shimadzu Co.). The column was eluted by using a linear gradient of solvent water (solvent A) and methanol (solvent B), starting with 30% of B (0−15 min) and increasing to 90% (15−75 min), held at 90% B (75−95), and decreasing to 30% of B (95−105 min) with a solvent flow rate of 1 mL/min and detection with a diode array detector. Chromatograms were recorded at 268 nm. The authentic standards of flavonoids were purchased from Extrasynthese Co., Genay, France. Pinobanksin, pinobanksin-3-acetate, and dimethylallylcaffeic acid were donated by Dr. E. Wollenweber, Darmstadt, Germany.

**Antiradical Activity Determination.** Free radical scavenging efficiency of the ethanolic extracts of propolis was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) (*15*−*18*) method described in refs *15*−*18*. Respective extracts of propolis samples, 20 $\mu$L, were mixed with 980 $\mu$L of methanol, and the mixture was added to 2 mL of DPPH solution (10 mg/L in 80% methanol). Five minutes later, the absorbance was measured at 517 nm. A reference sample was prepared with 1 mL of methanol. Reduction of DPPH by an antioxidant or by a radical species results in a loss of absorbance. Thus, the degree of decoloration of the solution indicates the scavenging activity of the added sample. The antiradical activity was calculated as a percentage of DPPH decoloration using the following equation:

$$\text{antiradical activity} = 100 \times (1 - \text{absorbance of sample/absorbance of reference})$$

**Antimicrobial Activity of Ethanolic Extracts of Propolis.** Examination of propolis sensitivity to *Staphylococcus aureus* ATCC 25923 was determined according to the method described in refs *19* and *20*. Actively growing nutrient broth cultures of *S. aureus* were inoculated in nutrient agar plates with sterile swabs, which were dipped in broth culture and applied to the extracts of propolis disk on the inoculated plate, and incubated overnight at 37 °C. The extracts of propolis were prepared by submerging 10 $\mu$L into Whatman filter paper no. 3 disks (5 × 1 mm) and dried under low vacuum at room temperature overnight and then incubating at 60 °C for 4 h.

**Analysis of Total Phenolic Content.** The concentration of total phenolics of ethanolic extracts of propolis was determined using the Folin−Ciocalteu reagent as described by Yu et al. (*18*). The reaction mixture contained 0.1 mL of the extracts, 0.5 mL of Folin−Ciocalteu reagent, and 1.5 mL of 20% sodium carbonate. The final volume was brought up to 10 mL with deionized water. After 2 h of reaction at room temperature, the absorption was measured at 760 nm and the phenolic content calculated using gallic acid as a standard. Results were expressed as milligrams per gram of propolis of gallic acid equivalents.

## RESULTS AND DISCUSSION

**Propolis Samples.** Twenty-five samples of propolis were collected from seven different regions in northern Argentina:

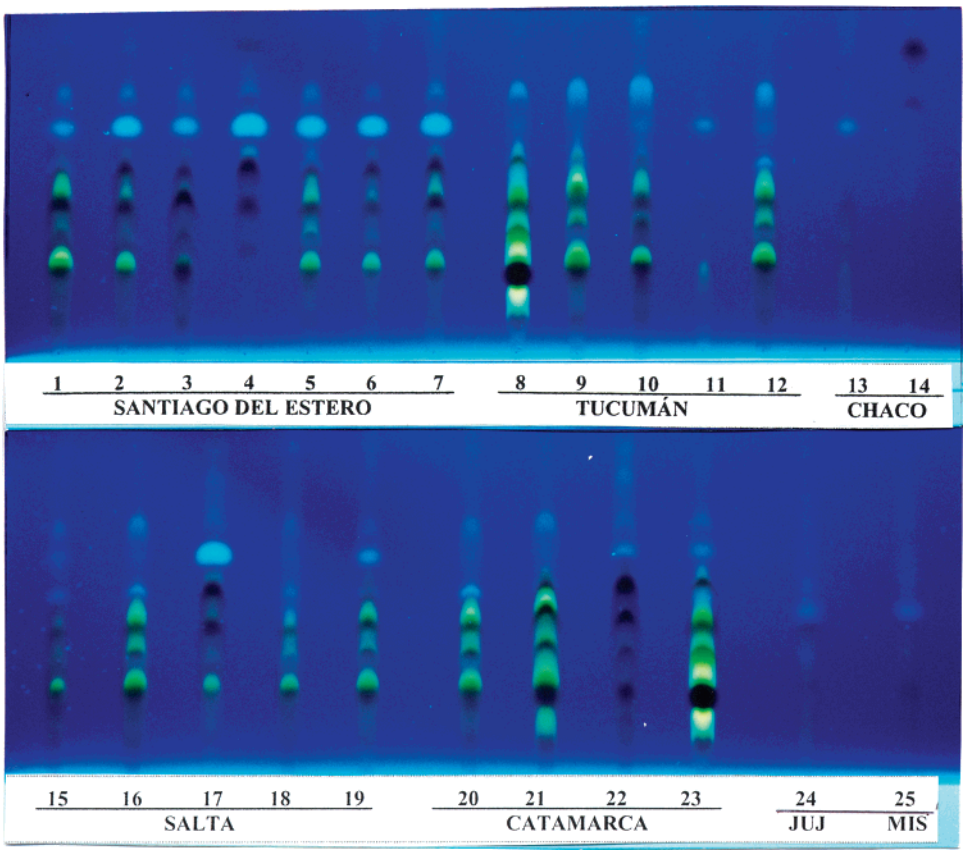



**Figure 2.** RPHPTLC of ethanolic extracts of propolis.

**Table 1.** Flavonoids and Other Chemical Constituents of Propolis[a]

| | | propolis sample | | | | | | |
|---|---|---|---|---|---|---|---|---|
| peak | compound | 3 (SE 3) | 4 (SE 4) | 8 (T 1) | 14 (CH 2) | 17 (S 3) | 19 (S 5) | 22 (C 3) |
| 1 | coumaric acid | 2.27 | 5.63 | 1.68 | 16.35 | 6.46 | 0.92 | 8.65 |
| 2 | ferulic acid | 0.79 | 2.14 | 6.42 | 0.51 | 2.41 | 0.76 | 4.36 |
| 3 | cinnamic acid | 0.92 | 1.57 | 8.26 | nd[b] | 1.63 | 0.54 | 1.83 |
| 4 | pinobanksin | 4.80 | 9.95 | 3.62 | nd | 10.29 | 2.86 | 6.22 |
| 5 | quercetin | 0.78 | 2.84 | 1.74 | nd | 2.29 | 0.75 | 1.03 |
| 6 | kaempferol | 0.77 | nd | 1.15 | nd | 0.18 | 0.70 | nd |
| 7 | apigenin | 3.05 | 3.32 | 1.83 | 1.37 | 1.04 | 1.19 | 2.85 |
| 8 | pinocembrin | 5.27 | 12.75 | 48.39 | nd | 14.83 | 7.32 | 17.27 |
| 9 | 1,1-dimethylallylcaffeic acid | 1.17 | nd | 1.74 | nd | 0.46 | 0.50 | 1.34 |
| 10 | chrysin | 2.58 | 0.33 | 5.82 | nd | 3.41 | 10.14 | 1.93 |
| 11 | galangin | 1.42 | nd | 0.39 | nd | 3.17 | 9.07 | 0.87 |
| 12 | kaempferide | 2.49 | nd | 2.28 | nd | 0.86 | 2.16 | 3.23 |
| 13 | tectochrysin | 2.32 | nd | 4.25 | nd | 1.91 | 4.45 | 1.67 |

[a] Milligrams of flavonoids per gram of propolis. [b] Not detected.

7 samples from Santiago del Estero (SE), 5 samples from Tucumán (T), 2 samples form Chaco, 5 samples from Salta (S), 4 samples from Catamarca (C), 1 sample from Jujuy (JUJ), and 1 sample from Misiones (MIS). Ethanolic extracts of propolis were prepared from all samples as described under Materials and Methods. The respective ethanolic extracts were examined for UV absorption spectra, RPHPTLC, RPHPLC, concentration of total phenolics, antimicrobial activity, and antiradical activity.

**Measurement of Absorption Spectra.** The absorption spectra of respective ethanolic extracts of propolis were measured, and the results are shown in **Figure 1**. All seven samples of propolis from Santiago del Estero (SE), three samples (9, 10, and 12) from Tucumán (T), all five samples (15−19) from Salta (S), and three samples (20−22) from Catamarca (C) showed one absorption peak with $\lambda_{max}$ at 290 nm. **Figure 1** shows only four representative samples, numbers 4, 17, 19, and 22. The absorption peak at 290 nm is similar to that of propolis group 3, which was collected in southern Brazil (*10*, *13*). In the case of samples 8 from Tucumán (T) and 23 from Catamarca (C), the absorption spectra were different as compared to the propolis that showed an absorption peak with $\lambda_{max}$ at 290, and these two samples had one broad band from 300 to 325 nm. Propolis samples 11, 13, 14, 24, and 25 did not show significant absorption spectra.

**RPHPTLC Profiles.** Ethanolic extracts of all the propolis samples were examined by RPHPTLC, and the results are shown in **Figure 2**. RPHPTLC revealed the same profiles except for samples 4, 8, 11, 13, 14, and 22−25; the profiles were similar to the RPHPTLC profile of propolis group 3 (G3) and 3 (poplar resinous exudates) from southern Brazil, which were published

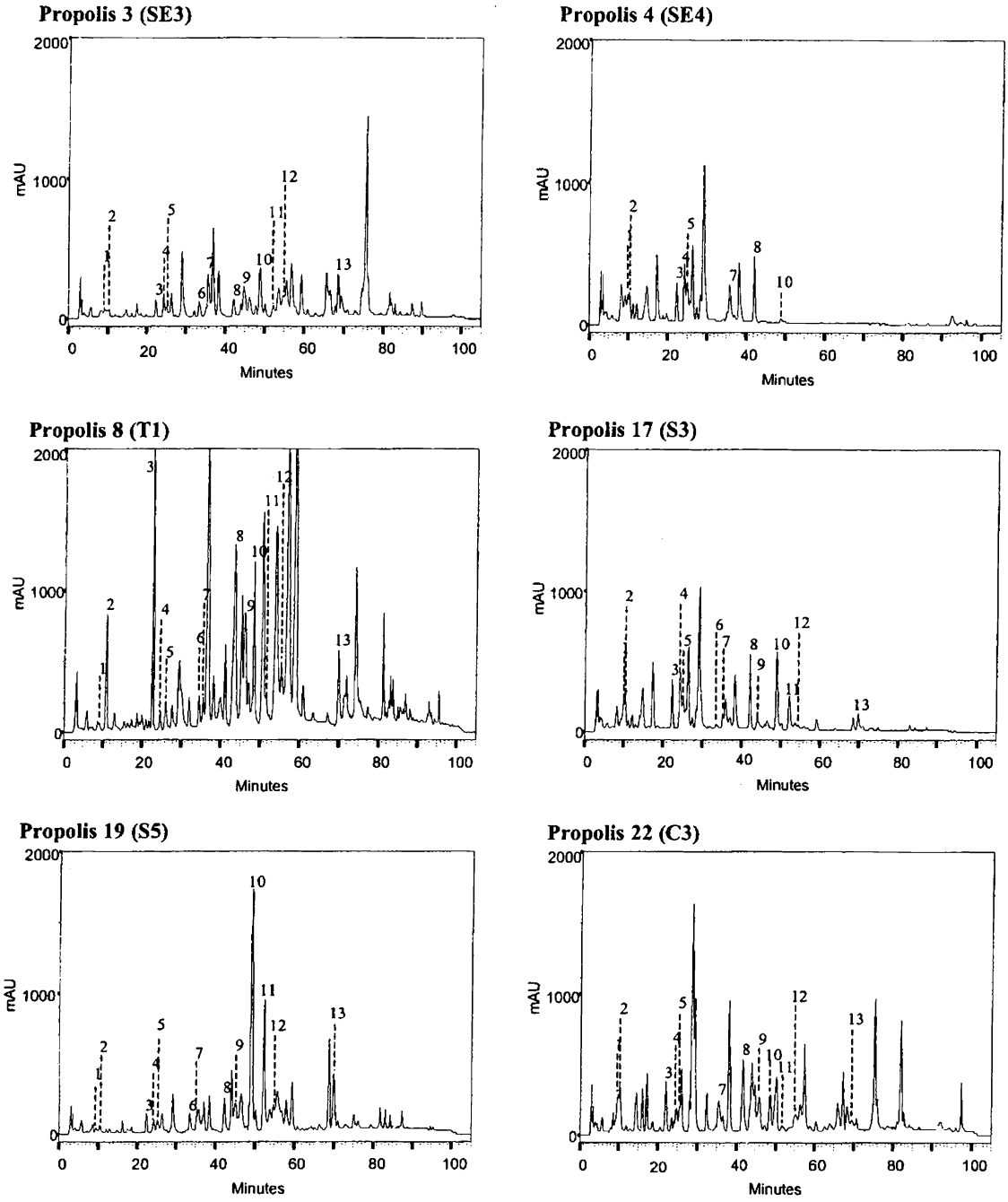
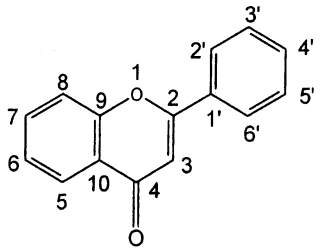



**Figure 3.** RPHPLC of ethanolic extracts of propolis.

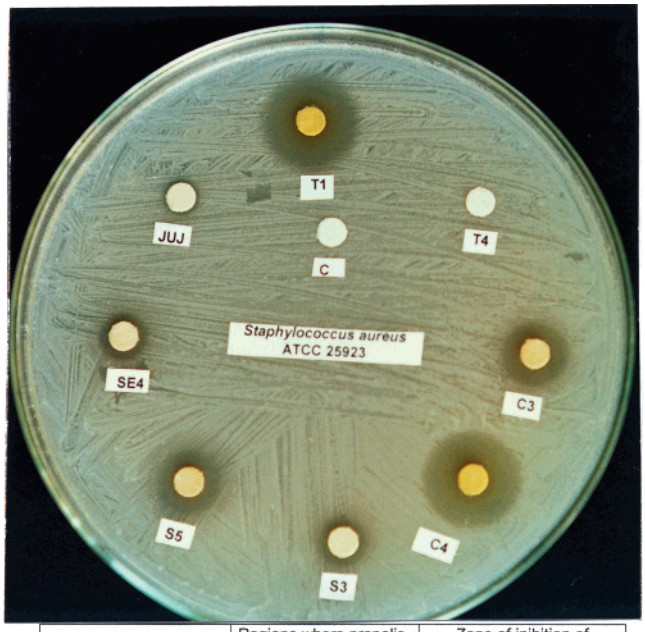
in refs *10*, *13*, and *14*. These results suggested that the botanical origin of these propolis samples is the poplar tree. Samples 4 and 22 showed somewhat similar profiles, but they seem to lack some chemical constituents. As shown in **Figure 1**, the absorption spectra of these samples showed one absorption peak with $\lambda_{max}$ at 290 nm, and the absorption peak at 290 nm is similar to that of propolis group 3, which was collected in southern Brazil (*10*, *13*), but sample 4 did not contain five chemical constituents, and sample 22 lacks only one compound as compared to the other samples as shown in **Table 1**. Samples 4 and 22 require further investigation. Samples 8 and 23 show clearer bands than the other samples. In accordance with the results of the absorption spectra and RPHPTLC for samples 8 and 23, their botanical origins seem to be different. Samples 11, 13, 14, 24, and 25 did not demonstrate clear profiles.

**Qualitative and Quantitative Comparisons of the Flavonoid Profiles.** The identification of flavonoids and other phenolic compounds was also carried out by direct HPLC on a C-18 reversed-phase column, and the absorption spectra resulting from diode array detection were used to distinguish peaks, using comparison with authentic flavonoid standards. The identified constituents were quantified, and the results are described in **Figure 4** and **Table 1**. Nine different flavonoids were identified and quantified in the samples of ethanolic extracts, which showed one absorption spectrum with $\lambda_{max}$ at 290 nm and demonstrated similar, but quantitatively different, RPHPTLC profiles. In the case of sample 4, only five different flavonoids were identified. Samples 8 and 23 contained higher concentrations of pinocembrin, 1,1-dimethylallylcaffeic acid, ferulic acid, and cinnamic acid as compared with other samples,




| Identified flavonoids described in Fig 3 | Attachment of hydroxyl and methyl groups to carbon atoms in basic nucleus | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 5 | 6 | 7 | 8 | 2' | 3' | 4' | 5' | 6' |
| 4. Pinobanksin | OH | OH | | OH | | | | | | |
| 5. Quercetin | OH | OH | | OH | | | OH | OH | | |
| 6. Kaempferol | OH | OH | | OH | | | | OH | | |
| 7. Apigenin | | OH | | OH | | | | OH | | |
| 8. Pinocembrin | | | | OH | | | | | | |
| 10. Chrysin | | OH | | OH | | | | | | |
| 11. Galangin | OH | OH | | OH | | | | | | |
| 12. Kaempferide | OH | OH | | OH | | | | $CH_3$ | | |
| 13. Tectochrysin | | OH | | $CH_3$ | | | | | | |

**Figure 4.** Antimicrobial activity of ethanolic extracts of propolis.




| No. of propolis samples | Regions where propolis was collected | Zone of inibition of microbial growth (mm) |
|---|---|---|
| 4 (SE4) | Santiago del Estero | 0,5 |
| 8 (T1) | Tucumán | 4,0 |
| 11 (T4) | Tucumán | 0 |
| 17 (S3) | Salta | 1,0 |
| 19 (S5) | Salta | 2,0 |
| 22 (C3) | Catamarca | 2,5 |
| 23 (C4) | Catamarca | 5,0 |
| 24 (JUJ) | Jujuy | 0 |
| Control (C) | | 0 |

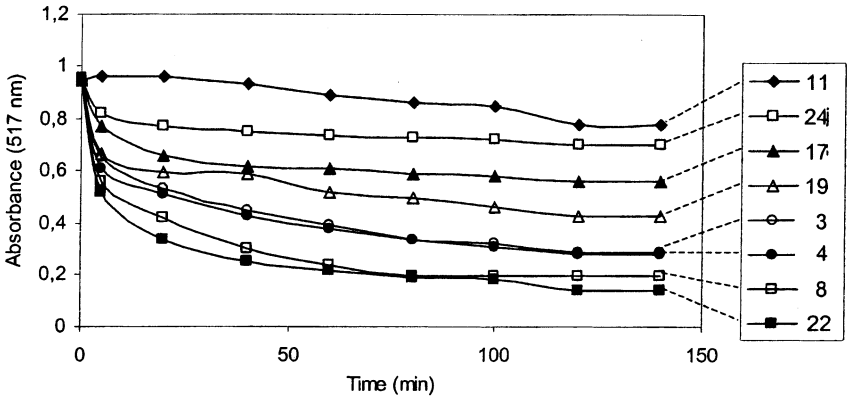
**Figure 5.** Antiradical activities of ethanolic extracts of propolis.

but sample 4 did not constitute 1,1-dimethylallylcaffeic acid, which is a common propolis constituent that bees collect from poplar resinous exudates and that has been implicated as a causative agent of contact allergies in bee keepers (*21*, *22*).

**Table 2.** Phenolic Content in Ethanolic Extracts of Propolis Samples

| propolis sample | location of collection | concn of phenolic content, mg/g of propolis |
|---|---|---|
| 1 | Santiago del Estero (SE 1) | 205.61 |
| 2 | Santiago del Estero (SE 2) | 200.75 |
| 3 | Santiago del Estero (SE 3) | 237.90 |
| 4 | Santiago del Estero (SE 4) | 226.10 |
| 5 | Santiago del Estero (SE 5) | 182.25 |
| 6 | Santiago del Estero (SE 6) | 190.34 |
| 7 | Santiago del Estero (SE 7) | 225.00 |
| 8 | Tucumán (T 1) | 358.80 |
| 9 | Tucumán (T 2) | 230.41 |
| 10 | Tucumán (T 3) | 194.12 |
| 11 | Tucumán (T 4) | 32.50 |
| 12 | Tucumán (T 5) | 202.50 |
| 13 | Chaco 1 | 58.23 |
| 14 | Chaco 2 | 67.60 |
| 15 | Salta (S 1) | 101.20 |
| 16 | Salta (S 2) | 198.60 |
| 17 | Salta (S 3) | 210.52 |
| 18 | Salta (S 4) | 197.00 |
| 19 | Salta (S 5) | 240.10 |
| 20 | Catamarca (C 1) | 200.76 |
| 21 | Catamarca (C 2) | 290.00 |
| 22 | Catamarca (C 3) | 295.23 |
| 23 | Catamarca (C 4) | 330.55 |
| 24 | Jujuy | 98.00 |
| 25 | Misiones | 89.00 |

**Antimicrobial Activity of Propolis.** Antimicrobial activity of all samples to *S. aureus* ATCC 25923 was measured according to the method described above, and the results are shown in **Figure 5**. Samples 8 and 23 demonstrated the highest inhibition of the bacterial growth as compared with other samples. These different patterns of sensitivity are due to different flavonoids in propolis (*19*). As shown in **Tables 1** and **2**, samples 8 and 23 contained highest concentrations of pinocembrin and phenolic content when compared with the other samples (sample 23 is not listed in **Table 1**, but its quantitative profile of flavonoids resembles that of sample 8). Previously, sensitivity of ethanolic extracts of propolis to *S. aureus* and *Streptococcus mutans* was examined in this laboratory, and it




The antiradical activity was calculated as a % of DPPH discoloration

| No. of Propolis samples | Antiradical Activity (%) |
|---|---|
| 11 (T4) | no reaction |
| 24 (JUJ) | 14,0 |
| 17 (S3) | 19,0 |
| 19 (S5) | 30,0 |
| 3 (SE3) | 32,0 |
| 4 (SE4) | 36,0 |
| 8 (T1) | 41,0 |
| 22 (C3) | 45,5 |

**Figure 6.** Antiradical activities of ethanolic extracts of porpolis.

was found that all propolis samples from various regions in Brazil showed inhibition of bacterial growth, but one of the samples (G3 propolis) from Rio Grande do Sul state demonstrated the highest inhibition of the bacteria. Therefore, analysis of flavonoids in this propolis was carried out, and it was found that the propolis contained the highest concentration of pinocembrin when compared with the others (*19*). However, samples 8 and 23 showed extremely higher antimicrobial activity than the other samples.

**Antiradical Activity of Propolis.** The physiological effects of propolis may be due to the presence of abundant phytochemicals including polyphenolics, mainly flavonoids. It is generally believed that these compounds have the property of antioxidation reactions and scavenging of free radicals (*17*, *23*). The aim of this experiment was to elucidate the relationship between the collected propolis and their ability of antioxidant properties. DPPH is free radical used for antioxidant activity. Reduction of DPPH by an antioxidant results in a loss of absorbance at 517 nm. Thus, the degree of discoloration of the solution indicates the scavenging efficiency of the added substances. The ethanolic extracts of propolis showed free radical scavenging activity against DPPH as shown in **Figure 6**. The antiradical activity (ARA) of propolis samples 8, 22, and 23 was significantly higher than that of other samples. (Samples 23 is not shown in **Figure 5** because the result is similar to that of sample 8.) It was also found that these samples contained higher concentrations of total phenolics than other samples as shown in **Table 2**.

**Conclusion.** Twenty-five samples of propolis were examined, and it was found that 16 samples showed similar profiles of absorption spectra, RPHPTLC, and qualitative flavonoids. These results are similar to propolis group 3 and poplar leaf resins that were collected in southern Brazil. Furthermore, the main phenolic components are pinobanksin, pinocembrin, chrysin, galangin, tectochrysin, and 1,1-dimethylallylcaffeic acid. Therefore, the main botanical origin of the propolis is the poplar tree. Qualitatively, the content of flavonoids in the majority of propolis samples collected in northern Argentina resembles those found in propolis from other temperate regions such as Europe, North America, and New Zealand (*1*, *8*, *9*). Propolis samples 8 and 23 showed the highest antimicrobial and antiradical activities, and the propolis also contained highest concentrations of pinocembrin and phenolic content. Presently, we are investigating further polyphenolic constituents and the botanical origin of these two samples of propolis.

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