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Effect of Seasonal Variations and Collection Form on Antioxidant Activity of Propolis from San Juan, Argentina

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ABSTRACT Propolis was included in the Argentine Food Code as a functional food. The chemical parameters and antioxidant properties of propolis samples from the same colonies of *Apis mellifera* in San Juan (Cuyo region, Western Argentine) were compared every month for 1 year using two collection methods. Chemical parameters were analyzed by the spectrophotometric method and fingerprinting using high-performance liquid chromatography with ultraviolet detection. The antioxidant activities of propolis samples were measured using model systems including the analysis of the scavenging activities for 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and the β-carotene bleaching assay. The results showed that propolis had a higher free radical scavenging and lipid peroxidation inhibitory capacity than butylated hydroxytoluene and quercetin, antioxidants used in the pharmaceutical and food industries. The concentration required to scavenge 50% of free radicals (SC_{50}) values differed depending on the sample collection month. Samples collected in November had the highest antioxidant capacity. In all cases, SC_{50} values of propolis samples obtained by scraping were similar to those collected from a wire mesh (5 μg/mL for ABTS and 20–30 μg/mL for DPPH radicals). A significant positive correlation was found between the antioxidant capacity and flavonoid content of each analyzed extract. The chemical profiles were very similar. Galangin (3,5,7-trihydroxyflavone), an antioxidant compound, was detected in all samples as a major compound. The chromatographic profile suggests that of *Baccharis* sp., which would be one of the botanical sources of propolis from western Argentina, and the content of galangin can be used as a parameter for evaluating propolis quality. Our results suggest that Argentine propolis from Cuyo is a promising source of bioactive compounds as ingredients for developing functional foods with a beneficial impact on oxidative stress.

KEY WORDS: • ***antioxidant activity*** • ***Argentine propolis*** • ***functional food*** • ***scavenging activity***

INTRODUCTION

THE ANTIOXIDANT COMPOUNDS are a group of natural products that can be found in vegetables, fruits, honey, propolis, and beverages derived from plants (tea, red wine) and in many dietary supplements or herbal remedies. These compounds are thought to be beneficial for human health and disease prevention, providing protection against the harmful effects of oxidative stress, which is related to the risk of cardiovascular disease, cancer, and other degenerative diseases. Propolis is a complex resinous material collected by honeybees from buds and the bark of plants. It contains a wide variety of phenolic compounds, mainly phenolic acids and flavonoids, amino acids, and terpenes.[1–6] Propolis has shown a variety of biological effects like antiviral and antimicrobial activity against many Gram-positive and Gram-negative bacteria, yeast, and fungi. It has also proved to have anticarcinogenic, anti-inflammatory, antioxidative, anesthetic, and cytostatic properties.[1,7–14] The chemical composition and biological properties of propolis have been studied extensively in Europe, but only a few reports can be found from Argentina. Analysis of propolis from Northwest Argentina showed evidence of antibacterial activity, antimutagenic activity, and free radical scavenging activity (RSA) in addition to a protective action against copper-mediated oxidative modification of lipids.[4,6,12–14] Recently the major chemical component responsible for the antibacterial and antimutagenic effect of Amaicha del Valle propolis (Tucumán, Argentina) has been demonstrated to be

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2′,4′-dihydroxychalcone.[12,13] Although propolis is a polyphenol concentrate of practically no nutritional value, it presents numerous functional properties. Hence, propolis has been incorporated (May 2008) as a dietary supplement into the Argentine Food Code by means of Resolution 357/08 of the Secretaría de Agricultura, Ganadería, Pesca y Alimentación and 94/08 of the Ministerio de Salud de la Nación.

The functional properties of propolis depend on its chemical constituents, which may vary according to season, geography, and plant sources. Hence, propolis from the Northwest, West, and South of Argentina would have different biological properties. The quality of propolis is intimately related to the methods of harvesting and storage, tasks performed by the beekeeper. The best-known collection methods in Argentina are scraping and wire meshing.

The purpose of the present study was to determine the effect of seasonal variations and harvesting form on chemical parameters and antioxidant capacity of propolis samples of hives from San Juan, Argentina.

MATERIALS AND METHODS

Reagents

All reagents used were of analytical grade or high-performance liquid chromatography (HPLC) grade.

Propolis collection

The samples tested were collected from beehives in Calingasta, San Juan, Argentina from April to December and January to March. The samples were hand-gathered by scraping and from wire meshes from hives of Red de Ensayos del INTA-PROAPI (Proyecto Integrado de Desarrollo Apícola). They were stored at −20°C. Sample collection date and environmental conditions in Calingasta are shown in Table 1 (31°22′S; 69°30′W).

Preparation of propolis ethanolic extract (PEE)

Once frozen at −20°C, propolis was ground and extracted with *n*-hexane first and with ethanol next (2 g of samples in 100 mL of each solvent with a Soxhlet apparatus).[15]

Preparation of plant extract

Buds and leaves of *Larrea* sp., *Cercidium* sp., *Eucalyptus* sp., *Populus* sp., and *Baccharis* sp. (10 g amounts) separately were extracted with 100 mL over an 8-hour period in a Soxhlet apparatus.

Phytochemical screening

Phenolic components of the different extracts (2.5 μg) were separated by thin-layer chromatography (TLC) (Kieselgel 60 F254 0.2 mm, Merck, Darmstadt, Germany). Chloroform: methanol (9.5:0.5, vol/vol), benzene:dioxane:acetic acid (9:2.5:0.4 by volume), and ethyl acetate:formic acid:glacial acetic acid:water (10:1.1:1.1:2.7 by volume) were used as development solvents. The separated components were visualized under ultraviolet (UV) light (254 and 365 nm, model UV 5L-58 Mineralight lamp, UVP, Inc., Upland, CA, USA) and sprayed with 1% ferric trichloride, 1% methanolic diphenylboric acid-β-ethylamino ester (NP), followed by 5% ethanolic polyethylene glycol (PEG),[16] or aluminum chloride for phenolic compounds. Methanolic potassium hydroxide was used for coumarins, Dragendorff's reagent for alkaloids, and anisaldehyde/sulfuric acid for steroids and terpenes.[17] UV-visible absorption spectra (200–420 nm) were obtained using a Beckman (Palo Alto, CA, USA) model DU 650 spectrophotometer.[18,19]

Analysis of propolis flavonoids and phenolic compounds by spectrometric methods

Total phenolic compound content was determined by the colorimetric method using the Folin-Ciocalteau reagent.[15,20] Absorbance of the resulting blue color was measured at 765 nm. Results were expressed as galangin equivalents.

Total flavone and flavonol content was measured by a spectrophotometric assay based on aluminum chloride complex formation.[21] Galangin was used as a standard.

Table 1. Date of Sample Collection by Scraping and Environmental Conditions in Calingasta, San Juan, Argentina and Results of Chemical Analysis of Samples

		Temperature (°C)			*Percentage*		
Collection date	*Sample number*	*Minimum*	*Maximum*	*Relative humidity (%)*	*Wax*	*Resins*	*Mechanical impurities*
April 2000	324	7.0	22.0	49.0	19.2	66.2	5.6
May 2000	326	2.0	16.0	55.0	17.5	68.2	4.3
June 2000	328	−2.0	13.0	54.8	27.0	62.8	5.0
July 2000	330	−3.8	17.8	44.7	20.4	65.8	5.6
August 2000	332	0.0	18.0	39.8	36.7	51.1	6.2
September 2000	334	0.5	20.5	54.0	32.6	56.0	5.5
October 2000	336	7.4	25.9	42.7	24.2	66.3	3.7
November 2000	338	7.4	26.0	38.8	20.6	65.9	7.2
December 2000	340	13.8	29.7	45.5	20.0	68.3	4.6
January 2001	342	16.0	34.0	40.0	6.1	88.7	3.1
February 2001	344	17.0	33.0	41.0	8.3	83.5	2.8
March 2001	346	17.0	29.0	60.0	27.7	67.6	5.1

Total flavonone and dihydroflavonone content was estimated using the 2,4-dinitrophenylhydrazine colorimetric method. Total flavonone and dihydroflavonone was calculated as naringenine from a calibration.[22]

Chemical composition by HPLC of propolis samples collected by two extraction methods and plant extracts

The PEE was processed by HPLC using an RP-18 column (4.6×250 mm; particle size, 5 μm). The column was eluted by using a gradient of solvent: water:acetic acid (19:1 vol/vol) (solvent A) and methanol (solvent B), starting with 30% B (0–15 minutes), reaching 90% B (15–75 minutes), remaining at 90% B (75–95 minutes), and then decreasing to 30% B (95–115 minutes). The solvent flow was 1 mL/minute. Detection of compounds was carried out at 280 nm and 340 nm and the identification by comparison with commercially available compounds dissolved in methanol (96%, vol/vol) for its retention time and UV spectral data.

Antioxidant activity (AA) determination

β-Carotene-linoleic acid assay. AA of propolis extracts was determined according to the β-carotene bleaching method. In brief, 1 mL of 0.2 mg/mL β-carotene dissolved in chloroform was added to round-bottom flasks (50 mL) containing 0.02 mL of linoleic acid and 0.2 mL of Tween-20. Each mixture was then dosed with 0.2 mL of the corresponding extract (until 40 μg of phenolic compounds) or the positive (butylated hydroxytoluene [BHT]) or negative (water and ethanol) control. After evaporation to dryness under vacuum at room temperature, oxygenated distilled water (25 mL) was added. The mixture was shaken for 2 minutes and then subjected to thermal autooxidation at 50°C for 60 minutes. Solution absorbance was monitored at 470 nm on a spectrophotometer (Beckman model DU-650) by taking measurements at 10-minute intervals, and the rate of bleaching of β-carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate.

AA was expressed as percentage AA and calculated with the following equation:

$$\text{AA } \% = 100 \times [1 - (A_0 - A_t / A_{00} - A_{0t})]$$

where A_0 is the initial absorbance at 470 nm of the emulsion at time 0, A_t is the absorbance of the tested plant extract at time t (10, 20, 30, and 60 minutes), A_{00} is absorbance at the beginning of incubation without extract, and A_{0t} is absorbance at time t without extract.

Quenching of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The hydrogen donor activity of extracts was measured by the DPPH method according to Yamaguchi *et al.*[23] In brief, 1.5 mL of DPPH solution (300 μM in 95% ethanol) was incubated with the samples (2.5–40 μg of phenolic compounds). The reaction mixture was shaken and incubated for 20 minutes at room temperature, and absorbance was measured at 515 nm against a blank. The free RSA was determined by comparison with ethanol control. Quercetin, ascorbic acid, and BHT were used as reference compounds.

The percentage of RSA was calculated using the following equation:

$$\text{RSA } \% = [(A_0 - A_s) / A_0] \times 100$$

where A_0 is the absorbance of the control and A_s is the absorbance of the samples at 515 nm. SC_{50} values denote the concentration of sample required to scavenge 50% of DPPH free radicals.

Total antioxidant capacity assay. The antioxidant capacity assay was carried out by the improved 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation ($ABTS^{\bullet+}$) method as described by Re *et al.*[24] $ABTS^{\bullet+}$ was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature (23°C) in the dark for 16 hours. $ABTS^{\bullet+}$ solution (1 mL; absorbance of 0.70 ± 0.02 at 734 nm) was added to 3.1 μg of the phenolic compound of each tested sample and mixed thoroughly. The reaction mixture was allowed to stand at room temperature, and the absorbance was recorded at 734 nm, 1 minute after initial mixing and up to 6 minutes. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC) (in μmol of Trolox equivalents/100 g dry weight of propolis) and SC_{50} values.

Autographic assay

For a rapid visualization of antiradical activity autographic assays were performed using $ABTS^{\bullet+}$ or DPPH radicals.[25] Extracts (3.1 μg) were applied on silica gel 60 F254 TLC plates (Merck). Mixtures of chloroform:methanol (9.5:0.5 vol/vol), benzene:dioxane:acetic acid (9:2.5:0.4 by volume), and ethyl acetate:formic acid:glacial acetic acid:water (10:1.1:1.1:2.7 by volume) were used as the mobile phase. Then, the plates were dried overnight and covered with 3 mL of soft medium (0.9% agar) containing 1 mL of $ABTS^{\bullet+}$ (7 mM ABTS and 2.45 mM potassium persulfate) or DPPH (1 mg/mL). Plates were incubated at room temperature for 1 minute in the dark. Active samples appeared as light spots against a green-blue or purple background for ABTS or DPPH assay, respectively. The antioxidant areas were compared with the ratio of fronts of the related spots on the TLC plate revealed with different reagents.

Statistical analysis

All measurements and the data were analyzed by analysis of variance.

RESULTS AND DISCUSSION

The chemical composition of propolis changes according to geographical origin, local flora, and collecting time. As a consequence, its biological activity is also diverse. Due to the variability of propolis, the aim of this study was to evaluate the AA of propolis samples gathered for 1 year

Table 2. Date of Sample Collection by Wire Mesh and Environmental Conditions in Calingasta, San Juan, Argentina and Results of Chemical Analysis of Samples

		Temperature (°C)			*Percentage*		
Collection date	*Sample number*	*Minimum*	*Maximum*	*Relative humidity (%)*	*Wax*	*Resins*	*Mechanical impurities*
April 2000	325	7.0	22.0	49.0	8.3	79.9	2.6
May 2000	327	2.0	16.0	55.0	9.9	76.2	4.4
June 2000	329	−2.0	13.0	54.8	5.3	85.9	3.2
July 2000	331	−3.8	17.8	44.7	3.6	80.1	3.1
August 2000	333	0.0	18.0	39.8	9.9	67.6	5.2
September 2000	335	0.5	20.5	54.0	7.3	83.2	2.9
October 2000	337	7.4	25.9	42.7	25.3	60.5	4.5
November 2000	339	7.4	26.0	38.8	37.1	57.1	3.9
December 2000	341	13.8	29.7	45.5	28.4	64.2	7.5
January 2001	343	16.0	34.0	40.0	27.7	67.4	3.4
February 2001	345	17.0	33.0	41.0	36.8	59.8	3.0
March 2001	347	17.0	29.0	60.0	37.7	58.0	2.9

from colonies of *Apis mellifera* in a specific region of Argentina at different collection times using two extraction methods (scraping and wire mesh). In this region, climatic conditions are extreme, with freezing thermometer marks all the year: mean annual temperature is −1°C, and daily temperature amplitudes are 20–25°C in summer. The climate is dry, with snow during fall and winter and drought in summer. The dominant vegetation is represented by *Larrea* sp., *Cercidium* sp., *Eucalyptus* sp., *Populus* sp., and *Baccharis* sp.

The assay was performed with propolis collected by scraping and wire meshing, but no significant differences were detected between them in the amount of propolis harvested.

Chemical analysis

Propolis cannot be used as a raw material because it must be purified by extraction with solvents. The PEE was analyzed by TLC and showed a positive reaction after treatment with NP/PEG reagent. The UV-visible spectra (200–600 nm) of sample dilutions showed a maximum in the region around 290 nm. This maximum is attributable to flavonoid content.[26] Bankova[27] proposed that poplar propolis quality is based on total flavone, flavanone, flavonol, and dihydroflavonol content and phenolic compounds because these parameters correlate better with biological activity and are more informative than the quantification of individual components. The results of the quantitative analysis demonstrated that San Juan propolis (Western Argentina) has a high flavonoid content (69–251 mg/g) similar to that of Northwest Argentina (120.0–200.0 mg/g)[6,12,14,28] but higher than other Argentine regions (Estepa Pampeana, Parque Chaqueño, and Patagonia Norte).[28]

According to our results, collection by scraping produced more wax and less resins, phenolic compounds, and flavonoids than by wire meshing until the spring (Tables 1–3). All propolis samples had a higher flavone and flavonol content

Table 3. Phenolic Compounds, Flavones and Flavonols, and Flavanones and Dihydroflavanones in Propolis Samples Collected by Scraping and Wire Mesh

	Percentage					
	Scraping			*Wire mesh*		
	Phenolic compounds	*Flavones and flavonols*	*Flavanones and dihydroflavanones*	*Phenolic compounds*	*Flavones and flavonols*	*Flavanones and dihydroflavanones*
April 2000	27.3	16.7	0.3	34.9	23.9	0.5
May 2000	27.5	17.2	0.6	34.0	23.7	0.6
June 2000	25.9	14.8	0.4	35.3	24.5	0.5
July 2000	22.7	18.9	0.5	37.0	20.8	0.8
August 2000	23.0	11.6	0.2	33.9	18.5	0.7
September 2000	24.7	14.3	0.2	35.0	25.1	0.3
October 2000	27.8	17.6	0.4	30.0	24.1	0.5
November 2000	28.8	13.9	0.3	28.0	6.9	0.4
December 2000	33.9	18.2	0.5	25.1	11.1	0.3
January 2001	35.8	23.8	1.1	31.2	16.0	0.7
February 2001	36.3	21.9	0.6	22.3	13.7	0.9
March 2001	24.7	15.8	0.7	23.0	11.7	2.6

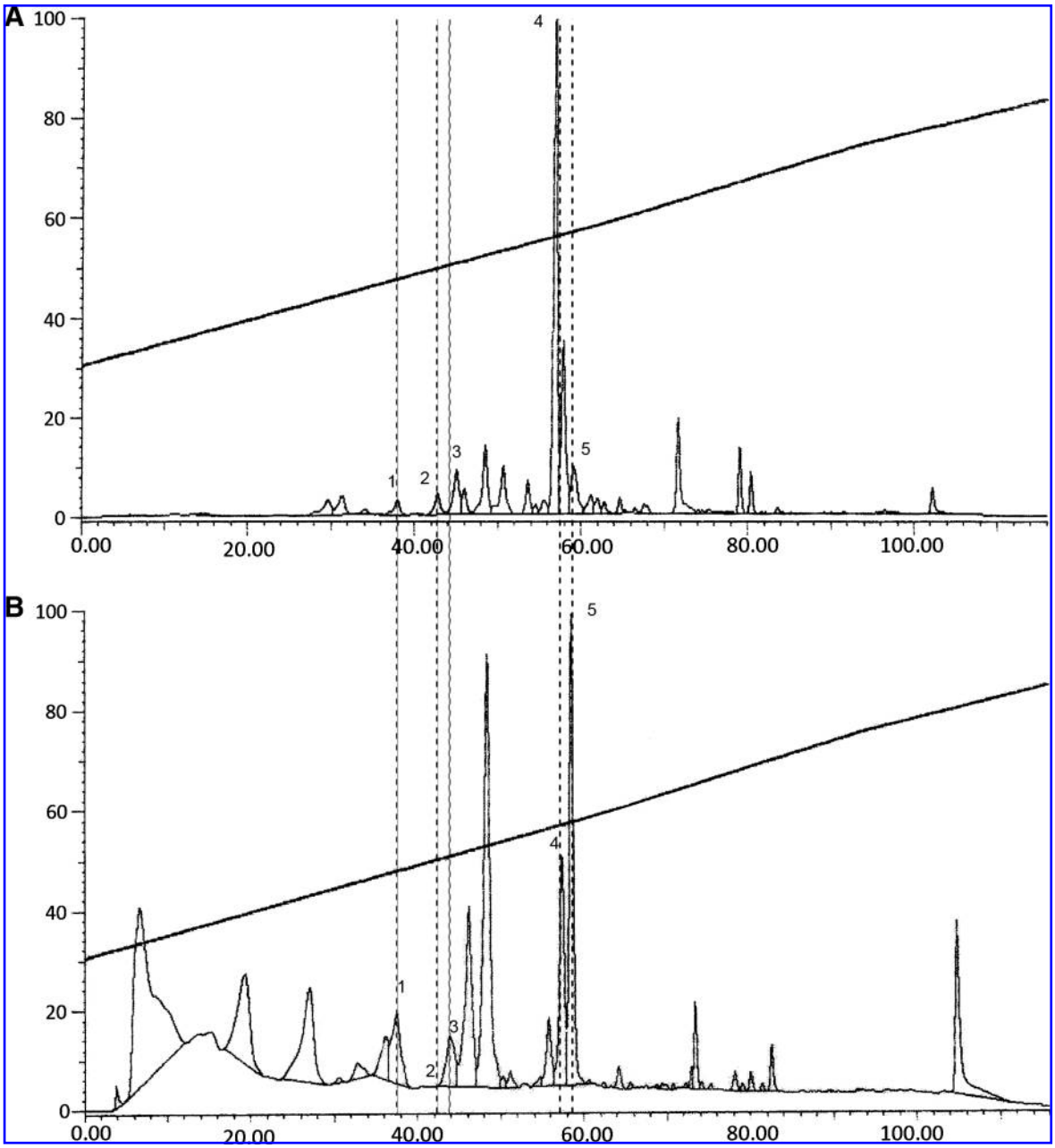

FIG. 1. Reverse-phase HPLC of propolis extracts collected by the scraping method in the months of (**A**) April and (**B**) November.

than flavanone and dihydroflavonones, thus indicating that they meet the IRAM-INTA norm[15] for propolis in nature (1.0 g of flavonoids/100 g of propolis).

The chromatograms of the PEE are shown in Figure 1. The chemical profiles obtained by both extraction methods are very similar. In these HPLC fingerprints, five peaks were identified. Number 4 was assigned as the reference peak because it was the highest and was identified as galangin. These studies thus suggest that galangina may be a phytochemical marker for the propolis from the Cuyo region in Argentina. Several functional properties have been ascribed to galangin. An anti-inflammatory effect of galangin has been attributed to suppression of eicosanoid synthesis through inhibition of cyclooxygenase-2 activity[29] and through its AA and RSA.[30,31] Results from *in vitro* and *in vivo* studies indicate that galangin with AA and free RSA is capable of modulating enzyme activities and suppressing the genotoxicity of chemicals.[32]

Botanical source of propolis

In the vicinity where the propolis was collected, five plant species were found and considered as a probable source of resin for propolis production. However, one plant species

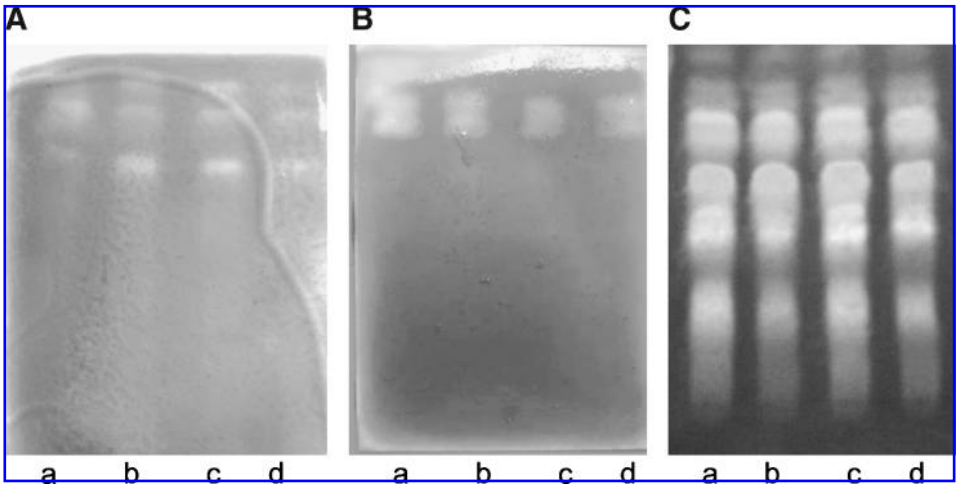

FIG. 2. Comparative autography of propolis extracts. Phenolic compounds (2.5 μg) of each extract/plate were separated by TLC (Kieselgel 60 F254, Merck) using benzene:dioxane:acetic acid (9:2.5:0.4 by volume) as eluant with samples collected as follows: lane a, July; lane b, November; lane c, January; and lane d, March. The plate was visualized by 365 nm UV light. Three milliliters of soft medium (0.6% brain-heart infusion agar) containing (**A**) DPPH or (**B**) ABTS was added. (**C**) The plate was revealed with NP/PEG.

showed more similitude to that of the analyzed propolis, and it was identified as *Baccharis* sp. (Fig. 1).

The voucher specimen was deposited in the herbarium of the Instituto de Estudios Vegetales de la Universidad de Tucumán, San Miguel de Tucumán, Argentina.

This is the first report about the potential botanical origin of Argentine propolis. The information about the botanical source is basic in quality control matters because it may help to develop links between types of propolis and specific biological activities and can be used to establish standards of quality of propolis.

AA

There is strong evidence that reactive oxygen species and free radicals play an important part in many degenerative diseases, such as cancer, atherosclerosis, and diabetes.[33] Formation of free radicals, such as superoxide anion radical and hydroxyl radicals, is an unavoidable consequence of respiration in aerobic organisms. These radicals are very unstable and react rapidly with other substances in the body, leading to cell or tissue injury. The body has its own defense system against reactive oxygen species, based on antioxidant enzymes and low-molecular-mass nonenzymatic antioxidant compounds. However, these defense systems are not effective enough to completely prevent damage. Thus, food supplements containing antioxidants may be used to help the human body to reduce oxidative damage. Several studies have described noteworthy AAs against the peroxidation of lipids or fatty acids or against free RSA of natural products. The DPPH assay evaluates the ability of hydrophilic antioxidants to scavenge free radicals, whereas $ABTS^{\bullet+}$ decoloration is useful to study the total AA of both lipophilic and hydrophilic antioxidants.

Contact autography, used for qualitative AA detection in chemical components separated by TLC, indicated that the propolis extracts have at least three well-defined antioxidant areas that correspond to flavonoids (Fig. 2).

Total AA

The propolis samples obtained from October to December showed the highest AA (86.785–80.958 μmol of TEAC/100 g of dry weight), whereas April–July values were 57,000–60,000 μmol of TEAC/100 g of dry weight. Experimental results demonstrated that the reaction with this radical is essentially completed within 1 minute. SC_{50} values of 6.25 μg of phenolic compounds/mL were obtained by both extraction methods (Fig. 3). The activity of propolis

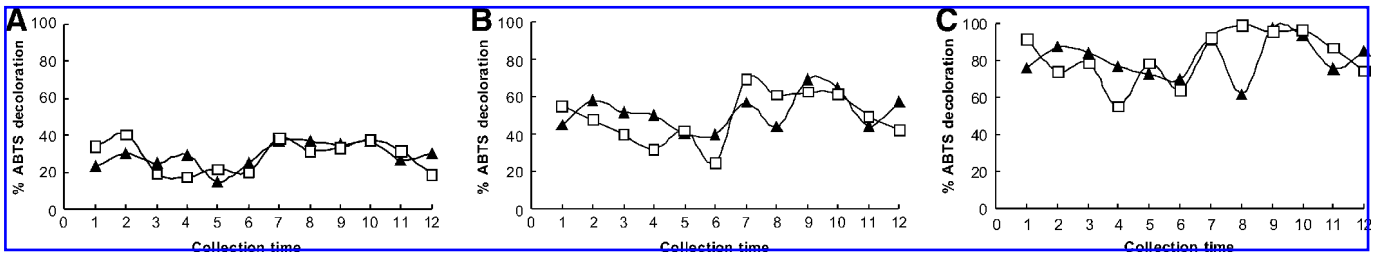

FIG. 3. Scavenging activity of $ABTS^{\bullet+}$ by (**A**) 2.5 μg, (**B**) 5 μg, or (**C**) 10 μg of propolis samples collected by scraping (□) or wire mesh (▲) during a 1-year period: (1) April, (2) May, (3) June, (4) July, (5) August, (6) September, (7) October, (8) November, (9) December, (10) January, (11) February, and (12) March.

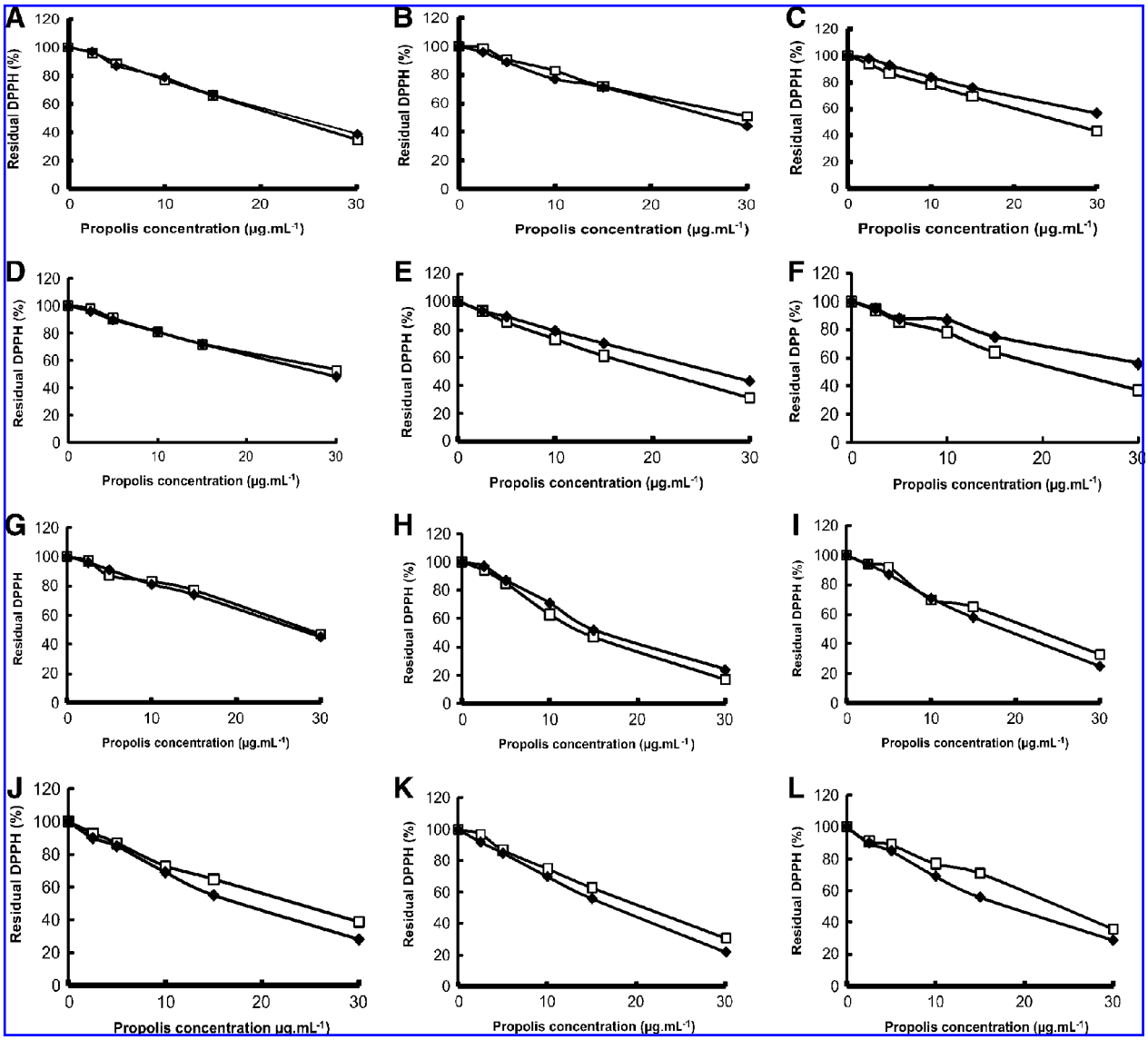

FIG. 4. DPPH RSA of propolis extracts collected by scraping (□) or wire mesh (◆) by month of collection: (**A**) April, (**B**) May, (**C**) June, (**D**) July, (**E**) August, (**F**) September, (**G**) October, (**H**) November, (**I**) December, (**J**) January, (**K**) February, and (**L**) March. Propolis extracts and the reference samples (quercetin and BHT) were used for the assay at final concentrations of 2.5–30 μg/mL. Measurements were carried out in triplicate.

samples was similar to that found for commercial galangin (5 μg/mL).

DPPH RSA

The percentage RSA values of the extracts were examined and compared with one another. Figure 4 shows the scavenging activity of various concentrations of propolis extract on DPPH degradation. All extracts inhibited DPPH absorption with values of up to 90%. The scavenging activity was very high, with SC_{50} values of 25–37.5 μg/mL (Fig. 5). The SC_{50} values and rate of DPPH radical scavenging were similar for both collection methods. The content of total phenolic compounds and flavone of each analyzed extract showed positive correlation with RSA ($R^2 = 0.80$). In order to characterize the antioxidative potency, the propolis samples were compared with natural and synthetic antioxidants. Considering these SC_{50} values, the activity of propolis samples was similar to that of natural antioxidants like ascorbic acid ($SC_{50} = 10\,\mu$g/mL), quercetin ($SC_{50} = 20\,\mu$g/mL), and galangin ($SC_{50} = 17\,\mu$g/mL) and more potent than BHT ($SC_{50} = 36\,\mu$g/mL).

AA on linoleic acid peroxidation

Table 4 shows the effect of propolis extract on linoleic acid peroxidation. The AA of 60 μg of propolis extract exhibited values from 13% to 72% depending on collection

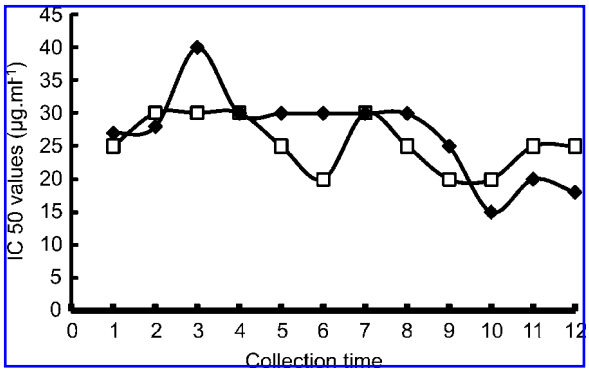

FIG. 5. SC_{50} values of DPPH scavenging of propolis extracts collected by scraping (□) or wire mesh (◆) by month of collection: (1) April, (2) May, (3) June, (4) July, (5) August, (6) September, (7) October, (8) November, (9) December, (10) January, (11) February, and (12) March. IC_{50}, 50% inhibitory concentration.

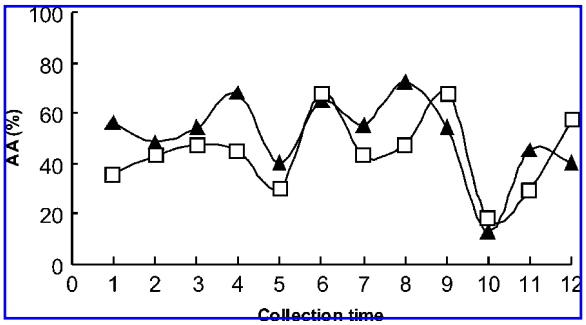

FIG. 6. AA of propolis extracts collected by scraping (□) or wire mesh (◆) in the β-carotene-linoleic acid system. Percentage AA values in the propolis extracts were determined according to the β-carotene bleaching method using 10 µg of flavonoid content/mL in the incubation mixture for 60 minutes. Measurements were carried out in triplicate. Propolis samples were collected for a 1-year period: (1) April, (2) May, (3) June, (4) July, (5) August, (6) September, (7) October, (8) November, (9) December, (10) January, (11) February, and (12) March.

time. The highest AA was obtained with samples collected by scraping in September–November. Samples gathered in January exhibited weak AA (Fig. 6). Flavones and flavonol content of each analyzed extract showed positive correlation with AA ($R^2 = 0.90$). Galangin, a major flavonol in propolis samples, protected against linoleic acid peroxidation with SC_{50} values of 216 µg/mL. This suggests that galangin can play a role in the AA of propolis extracts in a synergistic effect with other compounds.

CONCLUSIONS

The present study reveals that propolis extracts from San Juan in the Cuyo region of Argentina are natural antioxidants and free radical scavengers that may be exploited as biopreservatives in food applications as well as health supplements of functional food to alleviate oxidative stress. The best harvesting time for propolis samples is from October to December. Both scraping and mesh collection methods yielded similar results. Research is underway to identify other bioactive compounds.

TABLE 4. AA VALUES OF PROPOLIS EXTRACTS FROM SAMPLES COLLECTED BY SCRAPING AND WIRE MESH IN THE β-CAROTENE-LINOLEIC ACID SYSTEM

	AA (%)	
	Scraping	*Wire mesh*
April 2000	46 ± 12	36 ± 10
May 2000	48 ± 12	43 ± 5
June 2000	52 ± 14	41 ± 5
July 2000	68 ± 10	45 ± 5
August 2000	60 ± 10	30 ± 12
September 2000	65 ± 5	68 ± 10
October 2000	65 ± 5	53 ± 5
November 2000	72 ± 5	47 ± 5
December 2000	64 ± 10	68 ± 5
January 2001	13 ± 10	18 ± 10
February 2001	35 ± 12	29 ± 12
March 2001	40 ± 12	57 ± 10
BHT[a]	48 ± 10	

The percentage AAs in the propolis extracts were determined according to the β-carotene bleaching method using 12.5 µg of phenolic compounds in the incubation mixture for 60 minutes. BHT was used at a final concentration of 12.5 µg/mL. Measurements were carried out in triplicate. Data are mean ± SD values.

[a]Reference compound.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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