Study on Propolis Quality from China and Uruguay

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Propolis, Phenolic Constituents, ROO•-Scavenging Potential Activity

The composition, bacteriostatic and ROO•-scavenging potential activities of fifteen propolis samples from various botanic and geographic origins were determined to obtain objective information related to propolis quality. Variance analysis showed significant differences ($p \leq 0.05$) in the contents of polyphenols, flavonoids and active components between fresh and aged propolis. The state of the product (fresh or aged) could be differentiate by using flavonoid pattern and biological activities. A minimum propolis concentration of 80 μg/ml was required inhibit *Bacillus subtilis* and *Staphylococcus aureus* while 800 μg/ml was required to inhibit *Escherichia coli* using fresh propolis. Aged propolis inhibit *B. subtilis* and *S. aureus* at concentration of 100 μg/ml and *E. coli* at 1000 μg/ml. A minimum flavonoids percentage of 18 g/100 g and a maximum ROO•-scavenging potential activity of 4.3 μg/ml were determined in fresh propolis. Flavonoids levels in aged propolis were approximately 20% lower than in fresh propolis. A maximum flavonoids percentage of 19.8 g/100 g and a ROO•-scavenging potential activity between 5.7 to 6.4 μg/ml in aged propolis were quantified. Another objective was to assess the use of ROO•-scavenging potential activity in propolis quality.

Introduction

Generally, biological actions of propolis is attributed to the flavonoid components. Propolis is a natural resinous product, elaborated by honeybees (*Apis mellifera*), that contains a complex mixture of phenolic compounds. The components of the soluble fraction in water or organic solvent (e.g. ethyl alcohol) present pharmacological properties such as bacteriostatic, antimycotic, antiviral, and antiherpes activities (Giurcoaneanu *et al.*, 1988; Olinescu *et al.*, 1991; Amoros *et al.*, 1992; Dimov *et al.*, 1992; Marcucci, 1995; Matsuno, 1995; Burdock, 1998). Of 17 authentic plant phenolic tested, 9 compounds (including rutin, chlorogenic acid, vanillin, vanillic acid, neohesperidin, gallic acid, shikimic acid, rhamnetin, and kaempferol) showed remarkably high alkylperoxyl radical (ROO•) scavenging activity present in propolis (Pascual *et al.*, 1994; Basnet *et al.*, 1997). Thus, a diet rich in these radical scavengers would reduce the cancer-promoting action of ROO• (Sawa *et al.*, 1999). Propolis dietary treatment stimulated different enzyme hepatic phase I and phase II activities (Siess *et al.*, 1996). The daily human intake of these polyphenols from different food sources in the American average diet has been estimated to be 1 g or more, which is significant since flavonoids have been shown to influence a wide range of biological functions increasing in elderly men (Hertog *et al.*, 1992). Phenolic compounds in red grape wine have been shown to inhibit in vitro oxidation of human low-density lipoprotein (LDL) (Hertog *et al.*, 1993; Abu-Amsha *et al.*, 1996). The biological role of the flavonoids and polyphenols remains to be elucidated, but there is growing evidence that an increase in dietary levels of these constituents may be of long-term benefit to human health (Shaw *et al.*, 1997). The most important botanical sources for obtaining flavonoids by honeybees are poplars (*Populus* sp.), birches (*Betula* sp.), willows (*Salix* sp.), chesnut tree (*Aesculus hippocastanum*), elms (*Ulmus* sp.), pine trees (*Pinus* sp.), oaks (*Quercus* sp.), spruces (*Picea* sp.) and ashes (*Fraxinus* sp.) (Serra Bonvehí and Ventura Coll, 1994). The color of propolis varies depending on climate in which the propolis is produced. The samples from regions with a temperate climate were pale brown to dark brown in color, propolis from Australia and tropical zones were black, while Caribean propolis was dark violet (Warakomska and Maciejewicz, 1992). Due to the variability of propolis, the aim of the study was to: (1) evaluate the antioxidant and antimicrobial power of fresh and aged propolis, (2) assess the

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stability of the phenolics in propolis on storage, and (3) develop a marker of the deterioration to differentiate aged and fresh propolis.

Material and Methods

Propolis samples

Fifteen propolis samples obtained from various botanic taxa, different geographic origins and varying presentations (powder and raw) were analyzed (samples nos. 1 to 2, geographical origin Uruguay/presentation raw; samples nos 3 to 6, geographical origin Uruguay/presentation powder; samples nos. 7 to 15, geographical origin China/presentation powder). The origin and plant taxa that contributed to the propolis were Anhui province (China) [*Robinia pseudoacacia* L., *Populus* sp. (*Aigeiros* section), *Morus* sp., *Pyrus* sp., *Prunus* sp., *Ulmus* sp., *Salix* sp. and *Melia azederach*], and from Uruguay [*Eucalyptus globulus*, *Populus* sp., *Betula* sp. and *Salix* sp.]. Once in the laboratory, they were kept in darkness and at room temperature. Samples were either homogenized or pulverized if necessary and analyzed in triplicate.

Instrumentation

A Shimadzu model UV-160A 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 Waters Associate Model 996 photodiode array detector.

Reagents and standards

Solvents were analytical (Panreac, Barcelona, Spain) and HPLC (Merck KGAa, Darmstadt, Germany) grade. Ultrapure water (Milli-Q, Millipore) was prepared for chromatographic use. (+)-α-Tocopherol and L-ascorbic acid (Merck, Germany). tert-Butyl hydroperoxide (t-BuOOH), hemoglobin/ferrous, phenol red, ferulic and coumaric acids were obtained from Sigma Chemical Co. (St. Louis, MO, USA). 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 were from Fluka Chemika (Buchs, Switzerland). Finally, 3,4-dihydroxybenzoic acid, isorhamnetin, sinapic acid, naringin, and tectochrysin were obtained from Extrasynthèse (Genay, France).

Analytical procedures

The water content of each propolis sample was determined by drying for 2 h to constant weight in a conventional kiln at 105 °C, cooled to room temperature and placed in a dessicator. Ash was determined by incineration at 500–550 °C to a constant weight. The wax content was determined by extracting with petroleum ether (40–60 °C) in a Soxhlet extractor for 3 h. Resins and total balsams were extracted for 30 min with methanol at room temperature. Phenols in the extract were determined with Folin-Ciocalteau reagent (RFC). The sample (0.5 g) of finely ground and unwaxed propolis was extracted by agitating with 70% methanol (v/v). 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 mg/kg of gallic acid (Marigo, 1973). HPLC analysis of phenolic compounds was performed according Serra Bonvehí and Ventura Coll (1994), utilizing 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. Essential oils were determined by steam distillation according to the method of Godefroot *et al.* (1981). Antioxidant power was determined by decoloration times by using the potassium permanganate method of Vajonina and Dushkova (1975). The oxidation rate was expressed as the time (s) of decoloring 0.1 N $KMnO_4$ solution in aqueous acid medium.

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 (Griffiths, 1982). Bathochromic movement of band I (320–380 nm) and band II (240–270 nm) using hydroxylation, methylation and metallic complexation was determined using the method of Serra Bonvehí and Ventura Coll (1994), as well as co-chromatography with pertinent markers.

Bacteriostatic activities

The bacterial cultures maintained and cultivated for bacteriostatic activity tests include: (i) *Bacillus subtilis* CIP 155 was cultivated for 7 days at 37 °C on Antibiotic Medium 32, which is the same as Medium 1, except for the additional ingredient of 0.3 g $MnSO_4$ (National Formulary, 1991)(Difco 0243; Difco Laboratories, Detroit, MI). The final adjusted suspension was adjusted to 10% transmittance at 580 nm with a serum blank; (ii) *Staphylococcus aureus* CECT 435 and *Escherichia coli* CECT 434 were cultivated on Antibiotic Medium 1 (Difco 0263) for 24 h at 37 °C. The final suspension was adjusted to 45% transmittance at 580 nm (Serra Bonvehí *et al.*, 1994).

Growth measurement on solid medium

Five different concentrations of each sample were introduced on petri dishes containing Antibiotic Medium 1. Each petri dish was inoculated with one drop of the suspension of test microorganism. The pattern test was performed with tetracycline ethanolic solution (ethanol 40%, v/v) according to Serra Bonvehí and Escolà Jordà (1995). Dishes were incubated at 37 °C for 20 ± 2 h (National Formulary, 1991; Meresta and Meresta, 1983).

Bioassay of ROO• *scavenging potential of flavonoids*

Is based on the bactericidal effect of ROO• but not RO• (alkoxyl) or alkyl (R•) radicals, which do not kill bacteria. The culture medium (mannitol broth) used for the bioassay based on the bactericidal action of ROO• was prepared by adding D-mannitol (10 g) (Difco 0170175) and phenol red (35 mg) to nutrient broth (18 g of powder) (Difco 0003150) in 1 l of water. The pH was adjusted to 7.4, followed by sterilization (121 °C, 20 min) (Akaike *et al.*, 1995). *Staphylococcus aureus* strain 209P, Gram-positive bacteria, was used in one series of experiments. The reaction mixture was composed of the following: 0.1 ml of *S.aureus* at 10^7 cfu/ml, 0.1 ml of hemoglobin at 1 mg/ml, 0.1 ml of the solution containing a test compound (flavonoid pattern, D-ascorbic acid, (+) – α-tocopherol or propolis), and 0.6 mL of PBS [phosphate-buffered saline: 8.1 mM Na_2HPO_4 + 1.5 mM KH_2PO_4 + 2.7 mM KCl + 137 mM NaCl (pH 7.4)]. Flavonoid pattern and propolis phenolic were extracted in ethanolic solution (ethanol 40%, v/v), and ascorbic acid in PBS solution. To quantitative radical scavenging activity of α-tocopherol, one part of α-tocopherol was mixed 1.3 parts of 20% egg yolk lecithin in ethanol, followed by removal of ethanol *in vacuo*. This mixture was then diluted with PBS (Akaike *et al.*, 1992). The reaction was initiated by adding 0.1 ml of 0.2 M *t*-BuOOH to the reaction mixture mentioned above at 37 °C, and was allowed to stand for 30 min. After incubation in the ROO• generating system, the bacterial suspension was serially diluted on a 96-well plastic multiplate in mannitol broth containing phenol red followed by overnight incubation at 37 °C. Scavenging of ROO• by test compounds permits bacterial growth, accompanying production of acidic metabolites in the culture medium and color change of the medium. The lack of color change indicated the lowest concentration of test sample that could kill the bacteria (Akaike *et al.*, 1995).

Statistical analysis

The results were analyzed using analysis of variance and the mean values compared using Duncan' s multiple range test at 5% level, using the SPSS computer package (SPSS, 1990).

Results and Discussion

The determination of characteristic profiles of fresh and aged propolis can be obtained by the using the following parameters: total phenolic compounds, flavonoids, waxes, essential oils, ashes, resins and balsams, decoloration time, and bacteriostatic activity and ROO•-scavenging potential activity. Table I gives values of the composition and physicochemical parameters (decoloration time) in fresh propolis. Woisky and Salatino (1998) propose that the propolis quality be based on flavonoids and phenolic compounds because these compounds are the main bioactive propolis con-

Table I. Physicochemical composition (g/100 g) in fresh propolis.

Sample No.	Wax	Resins and balsams	Phenolic compounds	Moisture	Decoloration time [s]	Essential oils	Ash
1	12.9	78.3	21.1	2.4	5.5	0.78	2.87
2	14.1	76.5	20.8	2.5	6.2	0.93	3.10
3	11.6	80.3	23.6	2.3	5.3	1.07	2.92
4	17.1	74.8	19.1	2.1	8.7	1.10	3.30
5	13.2	78.5	22.9	2.6	5.8	0.69	3.65
6	13.7	78.1	22.8	2	6	0.78	4.07
7	15.1	76.9	22.5	2.1	6.8	1.28	3.18
8	16.2	73.4	21.8	2.5	7.2	0.81	2.78
9	17.8	73.7	20.7	2.2	7.8	1.17	3.22
10	19.4	72.8	20.5	2.0	9.3	0.77	2.76
11	15.1	77.6	21.6	2.1	6.3	0.91	2.60
12	16.3	75.6	20.7	2.7	6.8	1.05	2.87
13	12.3	79.5	23.7	2.6	5.3	0.57	3.10
14	13.7	78.6	22.8	2.8	6.3	0.69	3.25
15	13.2	78.9	21.6	2.6	6	0.74	2.93
x	14.8	76.9	21.7	2.37	6.6	0.89	3.11
SD	2.2	2.35	1.29	0.27	1.19	0.20	0.37

Number of samples, n= 15; replicates, r = 3.

stituents present in resins and balsams fraction. Significant amounts of wax components were detected (11.6 to 19.4 g/100 g). Wax is not bioactive but can be correlated to the degree of decoloration. Essential oil contents ranged between 0.57 and 1.28 g/100 g, in agreed with literature values (Verzar-Petri *et al.*, 1986). High wax percentages in propolis increased decoloration time (samples nos. 4, 8, 9 and 10). Total phenolic compounds ranged between 20.5 and 23.7 g/100 g in fresh propolis (Table II). Flavonoids were predominant in the phenolic fraction (> 83%), and 93% of the samples had flavonoid contents higher than 19 g/100 g which is lower than the 35 g/100 g reported by Bankova *et al.* (1982). Benzoic acids, cinnamic acids and esters, and flavonoids (flavonols, flavones, and flavanones) were the three main types of phenolic present in propolis. Concentrations higher than 1 g /100 g of the detected and identified flavonoids components were found for: i) the flavonols and flavones (rutin, quercetin, apigenin, isorhamnetin and acacetin); ii) the flavanones (pinocembrin). Most of the flavonoids identified were aglycons due to the fact that honeybees segregate glucosidase during propolis processing, which promoter the hydrolysis of glucosides to free aglycones (Serra Bonvehí and Ventura Coll, 1994). The results presented here provide evidence that propolis is very active *in vitro* against *Bacillus subtilis* and *Staphylococcus aureus*, whereas *Escherichia coli* was less susceptible (Table III). In general *in vitro* assays have revealed significant activity of propolis in relation to a wide spectrum of pathogenic bacteria, particularly gram-positive strains (Marcucci, 1995; Mirzoeva *et al.*, 1997). This microbiological parameter is proposed as a quality criterion for propolis freshness. The most active propolis samples against *B.subtilis* and *S.aureus* showed a minimum inhibitory concentration (MIC) around 80 μg/mL (Table III). Propolis where less effective against *E.coli* with MIC values ranging from 800 to 900 μg/ml as opposed to a tetracycline MIC value of 1.50 μg/ml against *B. subtilis* and *S. aureus* and 2 μg/ml against *E. coli*, which are in agreement with Serra Bonvehí and Escolà Jordà (1995). No individual propolis active compound surpassed the Pearson-Lee values ($p \leq 0.05$) indicating that the observed increase in bacteriostatic activities was probably due to a cascade effect of all flavonoids and other active components.

Some flavonoids and other phenolic compounds have been reported to show ROO•-scavenging activity (Cao *et al.*, 1997). According to the results shown in Table III, the ROO•-scavenging potential activity had an approximate average variability of ≤ 4.20 μg/ml in fresh propolis. The minimal effective dose of quercetin (test compound) was esti-

Table II. Phenolic components in fresh propolis (g/100 g).

Component	Sample No.																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	x	SD
Caffeic acid	0.10	0.08	–	0.08	0.11	0.16	0.07	0.10	–	0.09	–	–	–	0.11	0.07	0.07	0.05
Vanillin	1.18	1.23	1.08	0.98	1.56	1.28	1.38	1.50	1.07	1.19	1.69	1.71	1.19	1.53	1.89	1.30	0.41
Ferulic acid	0.56	0.49	0.78	0.64	0.49	0.58	0.84	0.68	0.77	0.38	0.49	1.08	0.59	0.82	0.38	0.64	0.19
Sinapic acid	0.08	0.12	0.34	–	0.08	0.12	0.32	0.29	0.16	0.31	0.22	–	0.14	0.22	0.17	0.17	0.11
m-Coumaric acid	0.38	0.45	0.64	0.21	0.38	0.54	0.48	0.73	0.28	0.18	0.54	0.76	0.48	0.28	0.72	0.47	0.19
Naringin	-	–	0.09	0.15	–	0.1	0.07	0.22	0.29	0.17	0.10	0.36	0.42	0.31	0.23	0.17	0.13
Rutin	2.34	1.89	2.89	3.08	3.21	2.86	2.37	3.23	3.67	3.26	2.97	2.78	3.47	3.09	3.78	2.99	0.51
4-Hydroxybenzoic	0.10	0.16	0.26	–	0.08	0.23	0.31	0.28	0.16	–	0.28	0.41	0.07	0.38	0.28	0.20	0.13
o-Cinnamic acid	–	0.08	0.13	–	0.21	0.14	0.09	0.10	–	0.25	0.38	0.07	0.16	0.11	0.27	0.13	0.11
Quercetin	1.37	1.28	0.96	0.76	1.07	1.38	0.81	0.73	1.21	1.67	0.82	0.69	0.58	0.70	1.28	1.02	0.33
Hesperitin	–	0.08	–	–	–	0.10	0.17	0.07	0.21	–	–	0.08	0.11	0.18	–	0.07	0.08
Pinobanskin	0.17	0.20	0.19	0.17	0.10	0.23	0.18	0.29	0.18	0.16	0.08	0.23	0.18	0.28	0.20	0.19	0.06
Kaempherol	0.48	0.56	0.72	0.39	0.87	1.07	0.57	0.49	0.83	0.39	1.07	0.58	0.39	0.67	0.28	0.62	0.25
Apigenin	4.08	3.89	4.56	5.08	4.56	3.89	4.89	5.34	3.08	3.89	4.56	4.29	5.29	3.87	3.56	4.32	0.65
Isorhamnetin	1.28	2.08	1.65	1.07	1.49	1.22	2.45	1.98	2.39	1.78	1.78	1.49	2.07	1.64	1.39	1.72	0.41
Galangin	0.48	0.67	0.72	0.35	0.48	0.29	0.78	0.44	0.39	0.51	0.63	0.33	0.52	0.67	0.89	0.54	0.18
Chrysin	0.15	0.08	–	0.26	0.08	–	0.17	0.10	–	0.07	0.18	0.11	0.07	–	0.17	0.10	0.08
Acacetin	5.87	4.87	6.28	4.93	5.64	6.08	4.97	3.78	4.56	4.39	5.45	4.78	6.34	5.81	4.45	5.21	0.77
Pinocembrin	2.56	1.76	1.45	0.97	2.20	2.43	1.56	1.39	1.16	1.38	0.79	1.63	1.82	1.16	0.84	1.54	0.54
Pinostrobin	0.18	0.08	0.14	0.21	0.10	0.07	–	0.28	0.17	0.14	0.07	–	0.1	0.17	0.14	0.12	0.08
Tectochrysin	0.58	0.72	0.93	1.07	1.13	0.68	0.94	0.71	0.59	1.23	0.84	0.69	0.52	1.41	0.45	0.83	0.28
Rhamnetin	0.56	0.78	0.35	0.26	0.34	0.19	0.08	0.21	0.42	0.08	–	0.22	0.14	1.07	0.67	0.36	0.30
Total	22.5	21.6	24.2	20.7	24.2	23.6	23.5	22.9	21.6	21.5	22.9	22.3	24.7	24.4	22.1	22.9	1.23

Number of samples, n = 15; replicates, r = 3.

Table III. Minimum inhibitory concentration and ROO•-scavenging potential activity in fresh and aged propolis (μg/ml).

Sample No.	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	ROO•-scavenging
1	80/90	80/90	800/900	3.7/5.7
2	100/110	100/110	900/1000	3.9/6.1
3	90/100	90/100	800/900	3.4/5.7
4	110/130	120/130	900/1000	4.1/6.4
5	80/90	80/90	800/900	3.7/5.7
6	90/100	90/100	800/1000	3.9/6.1
7	90/100	90/100	800/900	3.7/5.9
8	100/110	100/120	900/1000	3.9/6.1
9	120/130	120/130	900/1000	4.1/6.1
10	110/130	120/130	900/1000	4.1/5.7
11	90/100	90/100	800/900	3.7/5.7
12	90/110	90/110	900/1000	3.7/5.7
13	80/90	80/90	800/900	3.8/6.1
14	90/100	90/100	800/900	3.8/6.1
15	80/90	80/90	800/900	4/5.9
x	93/105	95/106	840/947	3.8/5.9
SD	12.3/14.6	14.6/15	50.7/51.6	0.20/0.23

Fresh propolis/Aged propolis; Number of samples, n = 15; replicates, r = 3.

mated to be 85 μM (final concentration), in the presence of 20 mM *t*-BuOOH and 0.1 mg/ml hemoglobin which shows that the bactericidal action of ROO• was neutralized. Vanillin, gallic acid, kaempherol, naringin, apigenin, and acacetin exhibited activity comparable to rutin and coincidal with Sawa *et al.* (1999). Results showed a minimal relative ROO•-scavenging activity of fresh propolis was approximately 60 times higher than quercetin (Fig. 1). Therefore, quantification of the ROO•-scavenging potential activity of each phenolic compound would allow assessment of the total antioxidant potency (amount x specific ROO•-scavenging potential activity of each flavonoid). Consequently, the activity varied greatly with fla-

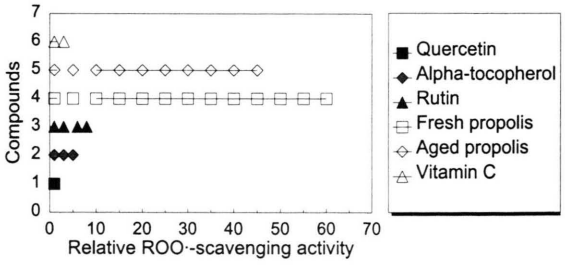

Fig. 1. Relative ROO•-scavenging potential activity based on a molar basis of various compounds expressed as a quercetin equivalent activity.

vonoid content and number of active components. The minimum presence of components in propolis should be assessed according to their antioxidant power and bacteriostatic activity. For these reasons a minimum number of active components should be present in propolis to assure its quality. We suggest that fresh propolis should contain a minimum of 15 components.

The evolution of phenolic compounds during the first year after collection of propolis was determined in all samples of the study in darkness and at room temperature (Table IV). A loss of total phenolic contents, between 17.5% and 25.1% of the initial value (20.8 ± 1.87%), was found, representing an average reduction of 1.73% per month at room temperature. Flavonoids levels in propolis were approximately 20% lower than in fresh product with a maximum percentage of 19.8 g/100 g. Significant differences ($p \leq 0.05$) could be noted between the flavonoid contents of fresh and aged propolis. Also, the loss of bacteriostatic activity and ROO•-scavenging activity were remarkable, with losses between 20–25% (Table III). In general the changes in bacteriostatic activity, and ROO•-scavenging capacity activity of the fresh and aged propolis, suggested that the activity reached a maximum in fresh propolis and decreased in aged propolis. Fig. 1 shows a minimal relative ROO•-scavenging potential activity 45 times higher than that quercetin equivalent in aged propolis (5.7 to 6.4 μg/ml), but 1.33 times lower than that in fresh propolis. However, no remarkable qualitative alternation in HPLC profiles of the flavonoids was observed. The Folin-Ciocalteu assay for total phenolics correlated well with the relative antioxidant activity measured in biossay of ROO•-scavenging potential activity of flavonoids. Akaike *et al.* (1995) indicated that the antioxidant substances having ROO•-scavenging potential can give excellent antibacterial effect induced by ROO•. The present study allowed to demostrate that propolis extracts are effective scavengers for ROO• generated in heating treatment (Rouit *et al.*, 1996). The minimal effective dose of propolis obtained from bioassay was remarkably greater than that of representative water-soluble antioxidants, e.g. 30 times greater than vitamin C acid based on a molar basis (Fig. 1). The examination of results reported in Cengarle *et al.* (1998), shows of the sardinian propolis extracts better antioxidant behaviour than the α-tocopherol coinciding with our results (11 times greater).

Table IV. Phenolic components in aged propolis (g/100 g).

	Sample No.																
Component	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	x	SD
Caffeic acid	–	–	–	–	–	0.08	–	–	–	–	–	–	–	–	–	–	–
Vanillin	0.87	0.76	0.68	0.56	1.18	0.67	1.05	1.22	0.81	0.79	1.24	1.39	0.85	1.19	1.43	0.98	0.28
Ferulic acid	0.46	0.34	0.56	0.49	0.35	0.41	0.69	0.54	0.59	0.27	0.31	0.88	0.36	0.67	0.20	0.47	0.18
Sinapic acid	–	0.07	0.23	–	–	0.06	0.13	0.09	0.05	0.23	0.11	–	0.07	0.13	0.08	0.08	0.07
m-Coumaric acid	0.25	0.33	0.46	0.08	0.19	0.33	0.28	0.51	0.10	0.06	0.36	0.48	0.35	0.11	0.58	0.30	0.17
Naringin	-	–	–	0.07	–	–	–	0.14	0.10	0.08	–	0.17	0.28	0.17	0.11	0.07	0.09
Rutin	1.98	1.71	2.53	2.81	2.96	2.57	2.03	2.80	3.26	2.94	2.67	2.43	3.18	2.79	3.26	2.66	0.47
4-Hydroxybenzoic	–	0.09	0.11	–	–	0.10	0.13	0.11	0.09	–	0.14	0.27	–	0.12	0.14	0.09	0.08
o-Cinnamic acid	–	–	0.07	–	0.11	0.07	–	–	–	0.09	0.16	–	0.06	–	0.16	0.05	0.06
Quercetin	0.98	0.87	0.75	0.48	0.81	0.93	0.71	0.58	0.94	1.38	0.68	0.51	0.38	0.52	0.86	0.76	0.25
Hesperitin	–	–	–	–	–	–	0.05	–	0.09	–	–	–	0.06	0.09	–	–	–
Pinobanskin	0.08	0.11	0.13	0.09	–	0.11	0.08	0.14	0.07	0.04	–	0.10	0.07	0.15	0.09	0.08	0.04
Kaempherol	0.23	0.40	0.51	0.22	0.68	0.91	0.38	0.28	0.63	0.13	0.84	0.32	0.17	0.41	0.12	0.42	0.25
Apigenin	3.58	3.36	4.19	3.95	3.67	3.54	4.40	4.87	2.67	3.49	4.28	3.89	4.38	3.49	3.28	3.80	0.56
Isorhamnetin	0.97	1.79	1.31	0.82	1.07	0.79	2.06	1.58	1.98	1.47	1.44	1.20	1.83	1.47	1.06	1.39	0.41
Galangin	0.31	0.53	0.44	0.18	0.27	0.11	0.49	0.27	0.21	0.39	0.49	0.18	0.22	0.42	0.33	0.32	0.13
Chrysin	0.07	–	–	0.11	–	–	0.06	–	–	–	0.07	–	–	–	0.07	–	–
Acacetin	5.22	4.11	5.78	4.31	5.17	4.98	3.19	3.27	4.19	3.79	4.93	4.23	5.84	5.26	3.89	4.54	0.84
Pinocembrin	2.23	1.44	1.12	0.67	1.79	2.08	1.23	0.91	0.79	0.89	0.56	1.16	1.34	0.82	0.67	1.18	0.52
Pinostrobin	0.07	–	0.05	0.11	–	–	–	0.14	0.07	0.08	–	–	–	0.06	0.08	0.04	0.05
Tectochrysin	0.29	0.55	0.69	0.96	0.81	0.48	0.61	0.52	0.38	0.85	0.61	0.40	0.33	1.08	0.36	0.59	0.24
Rhamnetin	0.41	0.52	0.17	0.11	0.21	0.07	–	0.11	0.27	–	–	0.13	0.06	0.78	0.48	0.22	0.23
Total	18	17	19.8	16	19.3	18.3	17.6	18.1	17.3	17	18.9	17.7	19.8	19.7	17.3	18.1	1.16

Number of samples, n = 15; replicates, r = 3.

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