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Antioxidant Activity and Constituents of Propolis Collected in Various Areas of Korea

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Propolis is a resinous substance collected by honeybees from various plant sources. The composition of propolis depends on time, vegetation, and the area of collection. This study examined the antioxidant activity of propolis from various areas of Korea: Chilgok, Cheongju, Geochang, Muju, Pocheon, and Sangju. Ethanol extracts of propolis (EEP) were prepared and evaluated for their antioxidant activity by β-carotene bleaching, 1,1-diphenyl-2-picrylhydrazyl free radical scavenging, and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assays. Furthermore, the major constituents in EEP were identified by high-performance liquid chromatography analysis with a photodiode array and mass spectrometric detection, and each component was quantitatively analyzed. EEP from Cheongju and Muju had relatively strong antioxidant activity accompanied by high total polyphenol contents. Propolis from Cheongju contained large amounts of antioxidative compounds, such as caffeic acid, kaempferol, and phenethyl caffeate. On the other hand, propolis from Pocheon had compounds not seen in propolis from other areas.

KEYWORDS: Antioxidant activity; propolis; free radical-scavenging activity; PDA; Korea

INTRODUCTION

Propolis, a folk medicine employed in treating various ailments, is a resinous substance collected by honeybees from the bud and bark of certain trees and plants and stored inside their hives. It has been used in folk medicine from ancient times in many countries and has been extensively studied in Eastern European countries (*1*). Recently, it has been reported to possess many biological activities such as antibacterial (*2*, *3*), antiviral (*2*, *4*), anti-inflammatory (*5*, *6*), anticancer (*7*, *8*), antifungal (*2*, *9*), and antitumoral (*10*) properties. For this reason, propolis is extensively used in food and beverages to improve health and prevent diseases such as inflammation, diabetes, heart disease, and cancer (*11*, *12*).

Propolis usually contains a variety of chemical compounds such as polyphenols (flavonoids, phenolic acids and their esters), terpenoids, steroids, and amino acids. Propolis contains >150 constituents and differs greatly due to variation in its geographical and botanical origins (*13*, *14*). Some of the observed biological activities might be attributed to the identified chemical constituents that partially stem from its high content of flavonoids. The compositions of propolis are qualitatively and quantitatively variable, depending on the vegetation at the area from which it was collected. Because of the geographical differences, propolis samples from Europe, South America, and Asia have different chemical compositions (*15*−*19*). Propolis from Europe and China contains many kinds of flavonoids and phenolic acid esters (*20*). In contrast, the major components in propolis of Brazilian origin are terpenoids and prenylated derivatives of *p*-coumaric acids (*21*−*24*). Due to the differences in their chemical compositions, the biological activities of various samples of propolis also differ with the geographic area. Kujumgiev et al. reported that propolis samples from different geographic origins were investigated for their antimicrobiological activity (*2*). Nieva Moreno et al. examined the free radical-scavenging activity of propolis extracts from several regions of Argentina and found that the correlation between high total flavonoid contents and free radical-scavenging activity was significant (*13*).

Studies on phenolic compounds and the antioxidant activity of propolis have been reported recently (*15*, *25*−*28*). Some of those papers have discussed the possibility of the prevention of several diseases by the antioxidant activity of propolis (*25*−*27*).

The chemical composition and biological activity of propolis have been studied extensively in many countries, but only a

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Figure 1. Collection sites of Korean propolis: **a**, Chilgok; **b**, Cheongju; **c**, Geochang; **d**, Muju; **e**, Pocheon; **f**, Sangju.

few reports can be found on Korean propolis. Analysis of Korean propolis extracts showed the presence of antimicrobial and pharmacological properties. Chee found that Korean propolis extract possessed high antifungal activity against *Cryptococcus neoformans* and *Candida albicans* (*29*). Song et al. reported that ethanol and ether extracts of Korean propolis produce estrogenic effects through activation of estrogen receptors (*30*). However, there are no reports on the antioxidant activity and chemical constituents of Korean propolis.

In this study, we investigated the in vitro antioxidant activity of the ethanol extracts of Korean propolis (EEP) from various geographic origins and analyzed the individual constituents in EEP. To our knowledge, this is the first report describing the antioxidant activity and constituents of propolis from various areas of Korea. We used three assay systems for evaluating Korean propolis: the inhibition of linoleic acid oxidation by β-carotene bleaching, the free radical-scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH), and the scavenging activity on 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation. Furthermore, we identified several compounds in EEP by high-performance liquid chromatography (HPLC) analysis with a photodiode array (PDA) and mass spectrometric (MS) detection and quantitatively analyzed each component.

MATERIALS AND METHODS

Materials. Caffeic acid (**1**), *p*-coumaric acid (**2**), and α-tocopherol (VE) were purchased from Sigma (St. Louis, MO). 3,4-Dimethoxycinnamic acid (**3**), pinobanksin 5-methyl ether (**4**), pinobanksin (**7**), cinnamylideneacetic acid (**8**), pinobanksin 3-acetate (**12**), and phenethyl caffeate (**13**) were isolated from the ethanol extract of Uruguayan propolis (*31*). Apigenin (**5**), kaempferol (**6**), chrysin (**9**), pinocembrin (**10**), galangin (**11**), and tectochrysin (**15**) were purchased from Funakoshi (Tokyo, Japan). Butylated hydroxytoluene (BHT), β-carotene, linoleic acid, potassium persulfate, and Tween 40 were purchased from Kanto Chemicals (Tokyo, Japan). ABTS, gallic acid, and DPPH were purchased from Wako Pure Chemicals Industries (Osaka, Japan).

Propolis samples were collected as the crude materials by beekeepers in various areas of Korea. **Figure 1** shows the collection sites of each sample. Crude propolis materials were extracted with ethanol at room temperature for 24 h. The ethanol suspension was separated by centrifugation, and the supernatant was concentrated under reduced pressure to give EEP.

Total Polyphenol and Flavonoid Contents. Total polyphenol contents in EEP were determined according to the Folin−Ciocalteu colorimetric method (*32*, *33*). EEP solution (0.5 mL) was mixed with 0.5 mL of the Folin−Ciocalteu reagent (Kanto Chemicals) and 0.5 mL of 10% Na_2CO_3, and the absorbance was measured at 760 nm after 1 h of incubation at room temperature. EEP samples were evaluated at the final concentration of 20 μg/mL. Total polyphenol contents were expressed as milligrams per gram of gallic acid equivalents.

Contents of flavonoid in EEP were determined according to the method of Woisky and Salatino with minor modifications (*34*). To 0.5 mL of EEP solution was added 0.5 mL of 2% $AlCl_3$ ethanol solution. After 1 h at room temperature, the absorbance was measured at 420 nm. EEP samples were evaluated at a final concentration of 20 μg/mL. Total flavonoid contents were calculated as quercetin (milligrams per gram) from a calibration curve.

Antioxidant Activity on Linoleic Acid Oxidation. This experiment was carried out according to the method of Emmons et al. with some modifications (*35*). β-Carotene (3 mg) was dissolved in 30 mL of chloroform, and 3 mL was added to 40 mg of linoleic acid and 400 mg of Tween 40. Chloroform was removed under a stream of nitrogen gas. Distilled water (100 mL) was added, and the solution was mixed well. Aliquots (3 mL) of the β-carotene/linoleic acid emulsion were mixed with 50 μL of EEP solution and incubated in a water bath at 50 °C. Oxidation of the emulsion was monitored spectrometrically by measuring absorbance at 470 nm over a 60-min period. The control sample contained 50 μL of solvent in place of the extract. The antioxidant activity is expressed as percent inhibition relative to the control after a 60-min incubation using the equation

$$AA = (DR_C|DR_S)/DR_C \times 100$$

where AA is the antioxidant activity, DR_C is the degradation rate of the control ($= \ln(a/b)/60$), DR_S is the degradation rate in the presence of the sample ($= \ln(a/b)/60$), a is the initial absorbance at time 0, and b is the absorbance at 60 min. EEP samples were evaluated at a final concentration of 10 μg/mL, and VE and BHT at 1 μg/mL were used as reference samples.

Free Radical-Scavenging Activity on DPPH. The effect of DPPH radical scavenging was evaluated according to the method of Okada and Okada with a slight modification (*36*). The assay mixture contained 2.7 mL of EEP solution and 0.3 mL of 2 mM DPPH ethanolic solution. After 15 min of incubation at room temperature in the dark, the absorbance was recorded at 517 nm. Results were expressed as percentage decrease with respect to control values. EEP samples were evaluated at a final concentration of 90 μg/mL, and VE and BHT at the same concentration were used as the reference samples.

Scavenging Activity of ABTS Radical Cation. The ABTS radical cation ($ABTS^{\bullet+}$) scavenging activity was measured according to the method described by Erel with some modifications (*37*). ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation was produced by reacting the ABTS stock solution with 2.45 mM potassium persulfate (final concentration). After the mixture was kept in the dark at room temperature for 12−16 h to allow the completion of radical generation, it was diluted with ethanol so that its absorbance was adjusted to 0.70 ± 0.02 at 734 nm. To determine the scavenging activity, 3 mL of diluted $ABTS^{\bullet+}$ solution was added to 20 μL of EEP solution, and the absorbance was measured at 734 nm 5 min after the initial mixing, using ethanol as the blank. The percentage inhibition was calculated by the equation

$$\%\ \text{inhibition} = (A_C|A_S)/A_C \times 100$$

where A_C is the absorbance of the control and A_S is the absorbance of the samples. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and VE were prepared as positive control samples. EEP samples were evaluated at a final concentration of 200 μg/mL, and Trolox and VE at 50 μg/mL were used as the reference samples.

HPLC Analysis with PDA and MS Detection. To identify and determine the constituents in EEP, we used HPLC with PDA and MS detection. EEP samples were dissolved in ethanol (5 mg/mL) and filtered with a 0.45 μm filter (German Sciences, Tokyo, Japan) prior to the injection of 10 μL into the HPLC system.

The HPLC system used was SI-1 (Shiseido, Tokyo, Japan) with a Capcell Pak ACR 120 (Shiseido) C18 column (2 × 250 mm i.d., 5

Table 1. Collection Sites and Total Polyphenol and Flavonoid Contents of EEP

propolis	collection site	total polyphenol[a] (mg/g of EEP)	flavonoid[b] (mg/g of EEP)
a	Chilgok	177 ± 3	95 ± 4
b	Cheongju	283 ± 5	136 ± 9
c	Geochang	138 ± 6	64 ± 6
d	Muju	228 ± 8	96 ± 4
e	Pocheon	85 ± 2	16 ± 2
f	Sangju	144 ± 2	66 ± 3

[a] Total polyphenol contents were determined by using the Folin–Ciocalteu method. Each value is the mean ± standard deviation. [b] Flavonoid contents were determined by $AlCl_3$ coloration. Each value is the mean ± standard deviation.

μm). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient was 20−60% B (0−60 min), 60−100% B (60−80 min), and 100% B (80−90 min) at a flow rate of 0.2 mL/min. For analysis by PDA detection, UV spectra were recorded from 195 to 650 nm at a rate of 0.8 spectrum/s and a resolution of 4.0 nm.

MS was performed on an LCQ ion trap mass spectrometer (Thermo Electron) equipped with an electrospray ionization (ESI) source. The operating parameters were as follows: source voltage, 5 kV; ES capillary voltage, −10 V; capillary temperature, 260 °C. All MS data were acquired in the negative ionization.

RESULTS AND DISCUSSION

Total Polyphenol and Flavonoid Contents of Various Propolis Samples. Propolis is commercially available as tablets made from ethanol extracts in many countries. We therefore examined the ethanol extracts from propolis samples obtained from various areas of Korea as shown in **Table 1**. All propolis samples, except that from Pocheon (**e**), were dark brown in color. The color of propolis from Pocheon (**e**) was bright yellow brown.

Table 1 shows the total polyphenol and flavonoid contents of EEP. The amounts of total polyphenol and flavonoid contents in Korean propolis varied widely and ranged from 85.3 to 282.9 mg/g of EEP and from 15.9 to 135.2 mg/g of EEP, respectively. EEP from Cheongju (**b**) and Muju (**d**) showed higher total polyphenol and flavonoid contents than those from other regions. Kumazawa et al. previously reported that the polyphenol content of EEP from Europe and China was ∼200−300 mg/g of EEP (*15*). The polyphenol contents in EEP from Cheongju (**b**) and Muju (**d**) were similar to those from Europe and China. However, the EEP samples from the areas other than Cheongju (**b**) and Muju (**d**) had a polyphenol content lower than those from Europe and China.

Phenolic compounds are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity (*38*). Propolis contains a wide variety of phenolic compounds, mainly flavonoids. The flavonoid content of propolis is attributed to the different preferred regional plants collected by honeybees. Contents of flavonoid and other phenolic substance have been suggested to play a preventive role in the development of cancer and heart disease (*38*). The Folin−Ciocalteu method and the $AlCl_3$ coloration are currently used to determine the total polyphenol and flavonoid contents, respectively (*39*, *40*). In the present study, we applied these methods to determine the total polyphenol and flavonoid contents of Korean propolis samples. These physicochemical methods are useful for evaluating various propolis samples because propolis contains many kinds of phenolics. Woisky and Salatino also evaluated propolis using these methods (*34*).

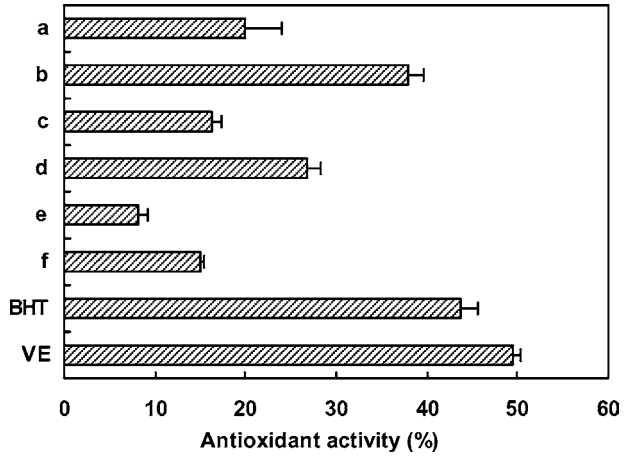


Figure 2. Antioxidant activity of EEP (**a**–**f**) of Korean propolis from various geographic origins in the β-carotene–linoleic acid system: **a**, Chilgok; **b**, Cheongju; **c**, Geochang; **d**, Muju; **e**, Pocheon; **f**, Sangju. Each EEP sample was used for the assay at a final concentration of 10 μg/mL. BHT and VE were used at a final concentration of 1 μg/mL. Measurements were carried out in triplicate. Means and standard deviations are indicated.

Effects of Various Propolis Samples on Linoleic Acid Oxidation. Figure 2 shows the antioxidant activity of various EEP samples determined by the β-carotene−linoleic acid system. The antioxidant assay using the discoloration of β-carotene is widely used, because β-carotene is extremely susceptible to free radical-mediated oxidation. β-Carotene is discolored easily by the oxidation of linoleic acid, because its double bonds are sensitive to oxidation (*41*, *42*). EEP samples were evaluated at a final concentration of 10 μg/mL for the assay, and VE and BHT were compared at 1 μg/mL under the same conditions.

As shown in **Figure 2**, EEP from Cheongju (**b**) had a stronger antioxidant activity than those from other regions. EEP from Muju (**d**) also had high antioxidant activity. The contents of both total polyphenol and flavonoid were high in EEP from Cheongju (**b**) and Muju (**d**). However, EEP from Pocheon (**e**), in which total polyphenol and flavonoid contents were small (**Table 1**), exhibited weak antioxidant activity. Phenolic compounds such as flavonoids are the type of antioxidant that possesses a strong inhibition effect against lipid oxidation through radical scavenging. Flavonoids have been reported to be the most abundant and most effective antioxidant in propolis (*43*−*45*). There are many papers concerning the antioxidant activity of the ethanol extract of propolis, which has been attributed to the high content of flavonoids in propolis (*46*−*48*).

DPPH Free Radical-Scavenging Activity of Various Propolis Samples. Because the free radical-scavenging activity of antioxidants is considered to be due to their hydrogen-donating ability, we used a method based on the reduction of DPPH, a stable free radical, to evaluate the antioxidant activity of various EEP samples (*48*, *49*). DPPH has been widely used to test the free radical-scavenging activity of various samples (*50*, *51*). We evaluated the free radical-scavenging activity of various EEP and the reference samples (VE and BHT) at a final concentration of 90 μg/mL (**Figure 3**).

As shown in **Figure 3**, the EEP sample from Cheongju (**b**) had strong DPPH free radical-scavenging activity, >90%. EEP from Cheongju (**b**) showed strong antioxidant activity, also in

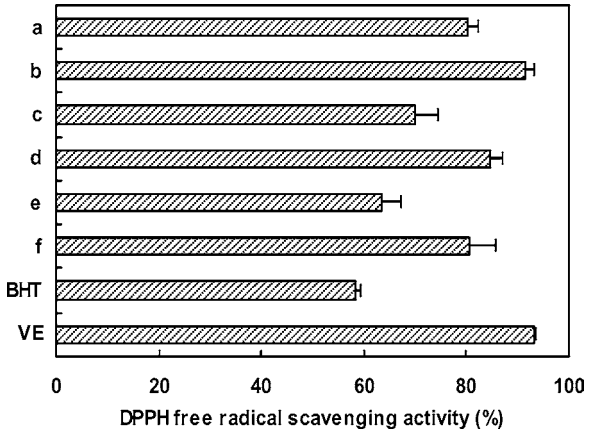


Figure 3. DPPH radical-scavenging activity of EEP (**a**–**f**) of Korean propolis from various geographic origins: **a**, Chilgok; **b**, Cheongju; **c**, Geochang; **d**, Muju; **e**, Pocheon; **f**, Sangju. EEP and reference samples (BHT and VE) were used for the assay at a final concentration of 90 μg/mL. Measurements were carried out in triplicate. Means and standard deviations are indicated.

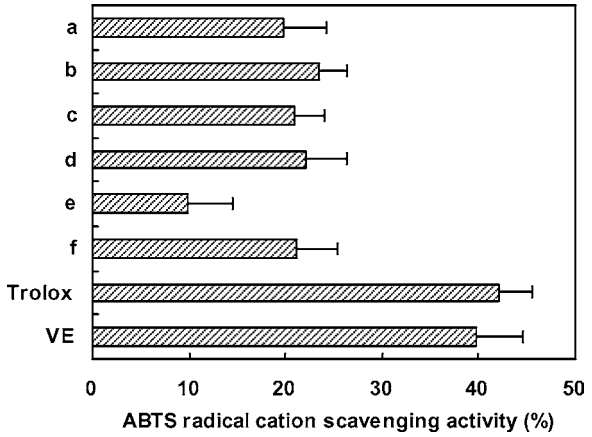
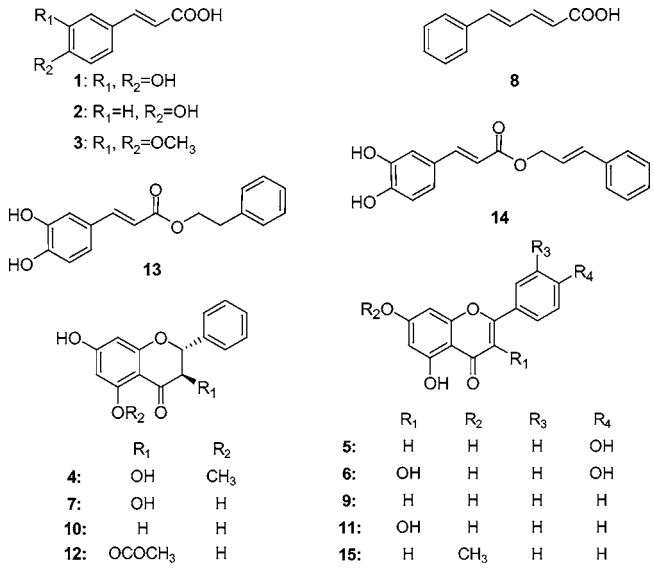


Figure 4. ABTS radical cation-scavenging activity of EEP (**a**–**f**) of Korean propolis from various geographic origins: **a**, Chilgok; **b**, Cheongju; **c**, Geochang; **d**, Muju; **e**, Pocheon; **f**, Sangju. Each EEP sample was used for the assay at a final concentration of 200 μg/mL. BHT and VE were used at a final concentration of 50 μg/mL. Measurements were carried out in triplicate. Means and standard deviations are indicated.

the assay system using the discoloration of β-carotene (**Figure 2**). EEP from Cheongju (**b**) and Muju (**d**) had high total polyphenol and flavonoid contents (**Table 1**), and the correlation between these contents and DPPH free radical-scavenging activity was significant (data not shown). EEP sample from Pocheon (**e**) had weak antioxidant activity in the assay system using the discoloration of β-carotene (**Figure 2**) and exhibited weak DPPH free radical-scavenging activity. The DPPH free radical-scavenging activity of all EEP samples was higher than that of BHT used as the positive control.

The DPPH free radical-scavenging activity shown in **Figure 3** seemed to correlate with the antioxidant activity shown in **Figure 2**. The propolis with high antioxidant activity also had high DPPH free radical-scavenging activity. However, more detailed qualitative and quantitative analyses of the compounds with antioxidant activity will be necessary to elucidate the antioxidant activity of propolis.

Effect of Various Propolis Samples on ABTS Radical Cation. The ABTS radical cation-scavenging activity of various EEP samples is shown in **Figure 4**. Most of them showed antioxidant activity. The ABTS radical cation decolorization



	R_1	R_2
4:	OH	CH_3
7:	OH	H
10:	H	H
12:	$OCOCH_3$	H

	R_1	R_2	R_3	R_4
5:	H	H	H	OH
6:	OH	H	H	OH
9:	H	H	H	H
11:	OH	H	H	H
15:	H	CH_3	H	H

Figure 5. Structures of the constituents identified from propolis: **1**, caffeic acid; **2**, *p*-coumaric acid; **3**, 3,4-dimethoxycinnamic acid; **4**, pinobanksin 5-methyl ether; **5**, apigenin; **6**, kaempferol; **7**, pinobanksin; **8**, cinnamylideneacetic acid; **9**, chrysin; **10**, pinocembrin; **11**, galangin; **12**, pinobanksin 3-acetate; **13**, phenethyl caffeate; **14**, cinnamyl caffeate; **15**, tectochrysin.

assay is a spectrophotometric method widely used for the assessment of antioxidant activity of various substances (*37*). The $ABTS^{\bullet+}$ is generated by the oxidation of ABTS with potassium persulfate and is reduced in the presence of such a hydrogen-donating antioxidant. EEP samples were evaluated at a final concentration of 200 μg/mL for the assay. Trolox and VE were compared at 50 μg/mL under the same conditions.

As shown in **Figure 4**, the EEP samples from Cheongju (**b**) had the highest activity. The percentage inhibitions of ABTS radical cation for all EEP samples except EEP of Pocheon (**e**) were similar, >20%. The ABTS radical cation-scavenging activity shown in **Figure 4** seemed to correlate with the antioxidant activity shown in **Figure 2**. The propolis with high antioxidant activity also had high ABTS radical cation-scavenging activity.

HPLC Analysis of Various Propolis Samples. We identified the major components in EEP samples by HPLC analysis by PDA and MS detection. The chemical structures of the compounds identified are shown in **Figure 5**. Previously, Kumazawa et al. isolated and identified 33 compounds: 18 flavonoids, 4 aromatic carboxylic acid, and 11 phenolic acid esters from Uruguayan propolis (*31*). Concerning the compounds that could not be obtained from commercial sources, we used those isolated from Uruguayan propolis as authentic compounds to identify each component.

Figure 6 shows the HPLC chromatograms of EEP samples **a**−**f**. The numbers **1**−**15** indicate the peaks identified by the HPLC analysis with PDA and MS detection. To identify each peak, UV spectra and the selected ion monitoring (SIM) of MS spectra of all peaks were compared with those of authentic samples. In the HPLC chromatograms of EEP from Pocheon (**e**), some peaks were observed after the retention time of 60 min. These peaks were not detected in other EEP samples. However, structural information of these peaks could not be obtained from PDA and MS analysis.

Fujimoto et al. analyzed various propolis samples from all over the world by UV and HPLC and classified the propolis into two groups according to the difference of their compo-

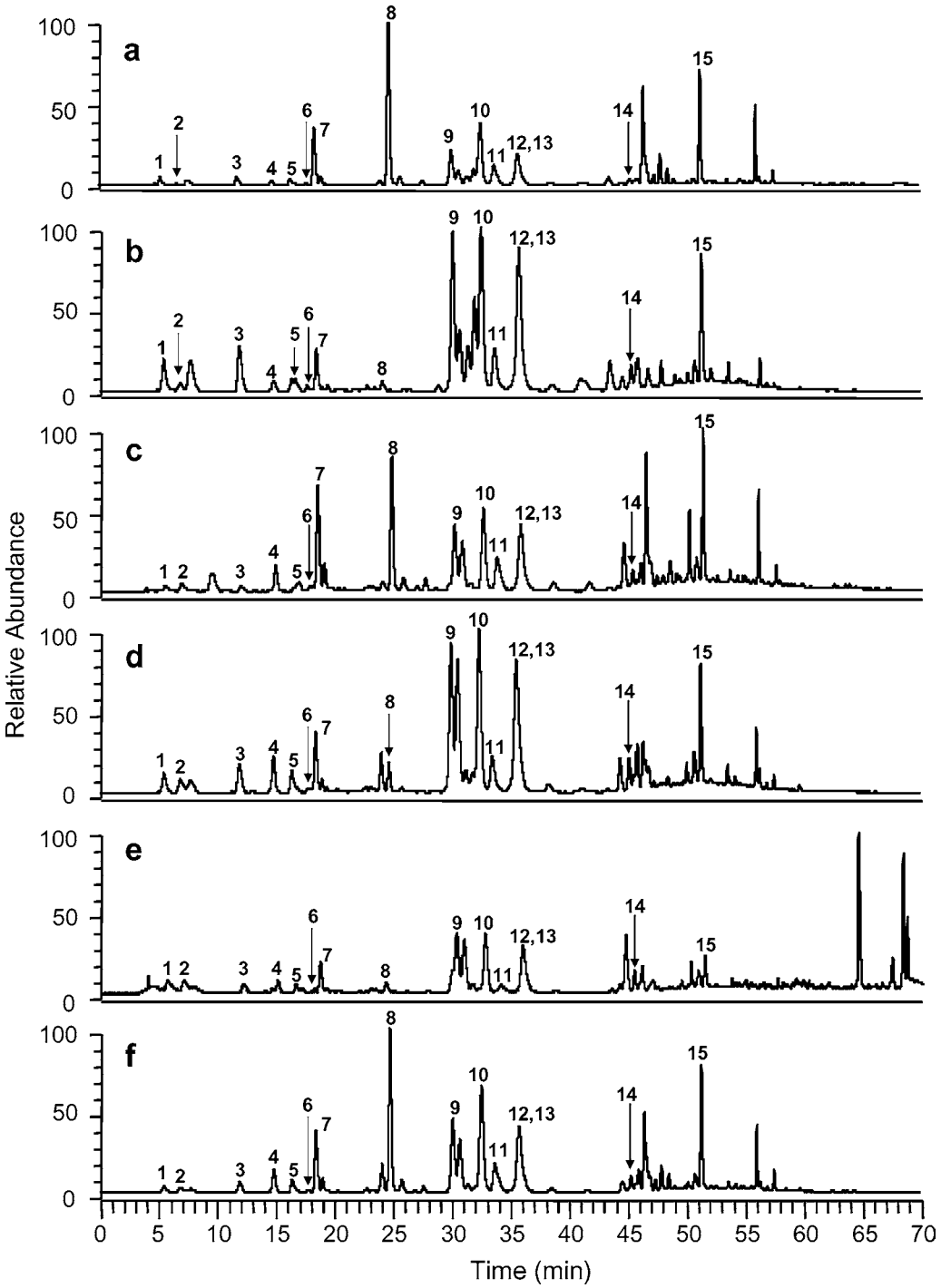


Figure 6. HPLC chromatograms of EEP (**a**–**f**) of Korean propolis from various geographic origins: **a**, Chilgok; **b**, Cheongju; **c**, Geochang; **d**, Muju; **e**, Pocheon; **f**, Sangju. The numbers in panels **a**–**f** represent the same compounds: **1**, caffeic acid; **2**, *p*-coumaric acid; **3**, 3,4-dimethoxycinnamic acid; **4**, pinobanksin 5-methyl ether; **5**, apigenin; **6**, kaempferol; **7**, pinobanksin; **8**, cinnamylideneacetic acid; **9**, chrysin; **10**, pinocembrin; **11**, galangin; **12**, pinobanksin 3-acetate; **13**, phenethyl caffeate; **14**, cinnamyl caffeate; **15**, tectochrysin.

nents: one is a Brazilian-type (*Baccharis*-type), and the other is a European-type (poplar-type) (*52*). Brazilian-type propolis is rich in *p*-coumaric acid derivatives and is found only in Brazil. On the other hand, European-type propolis is rich in flavonoids and is collected not only in Europe but also in China and other countries (*52*). The present study revealed that propolis samples from various areas of Korea were similar to European and Chinese propolis (poplar-type).

We quantitatively analyzed each component from the calibration curve of the HPLC chromatogram using authentic compounds. Because peaks **12** (pinobanksin 3-acetate) and **13** (phenethyl caffeate) overlapped on the HPLC chromatogram, they were not quantified by the HPLC analysis. Thus, for the quantitative analysis of peaks **12** and **13** we used the Xcalibur software (Thermo Electron) for quantitative determination based on the ion intensity of MS spectrum as described previously (*15*) (data not shown).

The results of the quantitative analysis of all EEP samples are shown in **Table 2**. Values are expressed as means of triplicate analyses for each sample. EEP from Cheongju (**b**) contained the largest amounts of **1** (1.7 mg/g of EEP), **3** (5.7 mg/g of EEP), **6** (2.3 mg/g of EEP), **9** (46.9 mg/g of EEP), **10**

Table 2. Content of the Constituents in EEP Samples

	content[a] (mg/g of EEP)					
	a	**b**	**c**	**d**	**e**	**f**
caffeic acid (**1**)	0.3 ± 0.0	1.7 ± 0.3	0.2 ± 0.0	0.6 ± 0.0	0.1 ± 0.0	0.4 ± 0.0
p-coumaric acid (**2**)	0.2 ± 0.0	0.7 ± 0.0	0.5 ± 0.1	0.7 ± 0.0	0.1 ± 0.1	0.6 ± 0.1
3,4-dimethoxycinnamic acid (**3**)	0.8 ± 0.1	5.7 ± 0.1	0.3 ± 0.0	1.9 ± 0.1	0. 1 ± 0.0	1.1 ± 0.2
pinobanksin 5-methyl ether (**4**)	1.0 ± 0.1	3.6 ± 0.3	3.7 ± 0.2	6.6 ± 0.1	0.2 ± 0.0	6.6 ± 0.3
apigenin (**5**)	1.9 ± 0.0	2.9 ± 0.1	0.4 ± 0.0	3.7 ± 0.7	0.1 ± 0.0	3.8 ± 0.3
kaempferol (**6**)	0.8 ± 0.1	2.3 ± 0.1	0.7 ± 0.0	0.8 ± 0.0	0.1 ± 0.0	1.1 ± 0.1
pinobanksin (**7**)	12.9 ± 1.0	12.6 ± 0.4	11.5 ± 0.6	9.1 ± 0.2	0.4 ± 0.0	15.4 ± 1.5
cinnamylideneacetic acid (**8**)	26.4 ± 1.9	2.8 ± 0.2	10.2 ± 0.4	3.5 ± 0.1	0.2 ± 0.1	29.4 ± 2.9
chrysin (**9**)	8.1 ± 0.6	46.9 ± 2.3	8.1 ± 0.4	22.7 ± 0.8	1.0 ± 0.1	18.5 ± 3.4
pinocembrin (**10**)	15.7 ± 1.2	52.5 ± 2.3	10.0 ± 0.8	27.3 ± 0.9	1.0 ± 0.1	30.0 ± 2.5
galangin (**11**)	9.6 ± 0.7	21.5 ± 1.3	10.1 ± 0.8	7.7 ± 0.1	0.4 ± 0.0	14.5 ± 1.4
pinobanksin 3-acetate (**12**)	9.9 ± 1.0	56.3 ± 2.3	10.5 ± 0.2	26.2 ± 0.4	1.1 ± 0.3	23.0 ± 2.5
phenethyl caffeate (**13**)	1.2 ± 0.1	7.7 ± 0.3	1.9 ± 1.0	6.3 ± 0.4	0.1 ± 0.1	6.1 ± 0.9
cinnamyl caffeate (**14**)	1.1 ± 0.1	3.3 ± 0.1	1.7 ± 0.1	3.6 ± 0.2	0.2 ± 0.1	2.9 ± 0.3
tectochrysin (**15**)	22.1 ± 1.5	41.3 ± 0.4	15.4 ± 0.9	19.3 ± 0.2	0.4 ± 0.0	27.0 ± 2.3

[a] Each value is the mean ± standard deviation.

(52.5 mg/g of EEP), **11** (21.5 mg/g of EEP), **12** (56.3 mg/g of EEP), **13** (7.7 mg/g of EEP), and **15** (41.3 mg/g of EEP) in all samples.

We identified 15 compounds from six kinds of Korean propolis and determined the quantitative value of each compound in the present study. The resinous excretions of the buds of the poplar tree are mentioned as the main sources of propolis from Europe, North and South America, and western Asia (*17*, *20*, *53*). Fujimoto et al. reported that the characteristic compounds of the propolis from China, Hungary, Bulgaria, Uruguay, and Argentina are pinocembrin, chrysin, galangin, and tectochrysin and that the source plant is *Populus* spp. (poplar) (*52*). Because we detected these compounds from the EEP samples from Korea, poplar may be one of the main source plants of these propolis samples.

EEP from Cheongju (**b**) showed strong antioxidant activity as mentioned above. Kumazawa et al. reported that compounds such as caffeic acid (**1**), quercetin, kaempferol (**6**), phenethyl caffeate (**13**), and cinnamyl caffeate (**14**) exhibited strong DPPH free radical-scavenging activity (*15*). As shown in **Table 2**, EEP from Cheongju (**b**) had a large amount of caffeic acid (**1**), kaempferol (**6**), and phenethyl caffeate (**13**). Thus, the high antioxidant activity of propolis from Cheongju (**b**) may be ascribable to these compounds.

In this study, we investigated the in vitro antioxidant activity of propolis samples from various areas of Korea. We identified the major constituents and quantitatively analyzed each component. The constituents and quantitative values in the propolis varied with the geographic origin. All Korean propolis samples except propolis from Pocheon had relatively similar antioxidant activities and constituents. Propolis from Pocheon, which is located in a northern region of South Korea and which is distant from the other sample areas, was quite different from that from other areas. To our knowledge, this is the first paper describing the diversity of Korean propolis. Further studies on the constituents and biological activities of Korean propolis are needed.

ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); BHT, butylated hydroxytoluene; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EEP, ethanol extracts of propolis; MS, mass spectrometry; PDA, photodiode array; VE, α-tocopherol.

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