Antioxidant Activity and Phenolic Constituents of Red Propolis from Shandong, China

Ai Hatano\textsuperscript{1}, Takashi Nonaka\textsuperscript{1}, Megumi Yoshino\textsuperscript{1}, Mok-Ryeon Ahn\textsuperscript{2}, Shigemi Tazawa\textsuperscript{3}, Yoko Araki\textsuperscript{3} and Shigenori Kumazawa\textsuperscript{1*}

\textsuperscript{1}Department of Food and Nutritional Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
\textsuperscript{2}Department of Food Science and Nutrition, Dong-A University, 840 Hadan 2-dong, Saka-gu, Busan 604-714, Republic of Korea
\textsuperscript{3}API Company Limited, Nagaragawa Research Center, 692-3 Yamasaki, Nagara, Gifu 502-0071, Japan

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Propolis is a resinous substance collected by honeybees from various plant sources and whose composition depends on the area of collection. This study examined the antioxidant activity of red propolis from Shandong, China. Ethanol extracts of propolis (EEP) were prepared and evaluated for their antioxidant activities using four assays: 2,2-diphenyl-1-picrylhydrazyl free radical-scavenging, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization, ferric ion reducing antioxidant and oxygen radical absorbance capacity assays. Red propolis from Shandong, China showed strong antioxidant activity across all assay systems used. Furthermore, the major constituents in EEP from Shandong, China were identified by HPLC analysis using photodiode array detection, and each component was quantitatively analyzed. In total, 17 compounds were identified: 9 flavonoids, 5 aromatic carboxylic acids and 3 phenolic acid esters. Red propolis from Shandong, China contains a large amount of the antioxidant compounds galangin and phenethyl caffeate (101.6 and 32.7 mg/g of EEP, respectively).

Keywords: propolis, red, antioxidant, phenolics, Shandong, China

Introduction

Propolis is a resinous substance that honeybees forage from the buds and exudates of certain plants and is stored by the bees inside the beehive (Bankova et al., 2000; Marcucci, 1995). It has been used in folk medicine from ancient times in many countries and has been extensively studied in Eastern European countries (Bankova et al., 2000; Castaldo and Capasso, 2002). Recently, propolis was reported to possess important biological activities such as antibacterial (Kartal et al., 2003; Kujumgiev et al., 1999), antiviral (Amoros et al., 1994; Kujumgiev et al., 1999), anti-inflammatory (Paulino et al., 2008; Strehl et al., 1994), anticancer (Kumoto et al., 2001; Matsumo, 1995), antifungal (Kujumgiev et al., 1999; Murad et al., 2002), and antitumoral (Ikeno et al., 1991) 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 (Banskota et al., 2001; Burdock, 1998).

Propolis usually contains a variety of chemical compounds such as polyphenols (flavonoids, phenolic acids and their esters), terpenoids, steroids and amino acids. Propolis is composed of more than 150 compounds, which differ greatly depending on the geographical and botanical origin of propolis (Bonvehí and Coll, 1994; Nieva Moreno et al., 2000). Some of the observed biological activities might be attributable to identified chemical constituents, in particular its high content of polyphenols. The composition of propolis varies both qualitatively and quantitatively, depending on the vegetation in the area from which it was collected. Thus, propolis from Europe, South America and Asia has different chemical compositions (Bankova et al., 1992; Kumazawa et al., 2004a; Kumazawa et al., 2004b; Velikova et al., 2001). Propolis from Europe and China (poplar origin), contains many different flavonoids and phenolic acid esters. In contrast, the major components of propolis from southern east Brazil (Baccharis origin) are terpenoids and prenylated derivatives of p-coumaric acids (Kumazawa et al., 2003; Marcucci and Bankova, 1999; Tazawa et al., 1998; Tazawa et al., 1999). Due to the differences in their chemical compositions, the biological activities of various samples of propolis also differ with their geographic origin.

*To whom correspondence should be addressed.
E-mail: kumazawa@u-shizuoka-ken.ac.jp
Previously, we reported prenylated flavonoids with potent antioxidant activity from propolis collected in Okinawa, Japan (Kumazawa et al., 2004a; Kumazawa et al., 2007). Some of these flavonoids were also isolated from Taiwanese propolis (Chen et al., 2008), although Okinawan propolis contains higher amounts of prenylated flavonoids than Taiwanese propolis (Kumazawa et al., 2007). Further, we found that the plant origin of Okinawan propolis is Macaranga tanarius (Kumazawa et al., 2008).

Recently, red propolis has been collected in Cuba and in northern Brazil (Alencar et al., 2007; Cuesta-Rubio et al., 2007; Daugsch et al., 2008), and we have found that a pale red propolis is also collected in Shandong, China. The red propolis from Brazil has been reported to have high antioxidant activity (Daugsch et al., 2008), as has the propolis from Shandong, China (Izuta et al., 2009). However, there are no studies on the relationship between the antioxidant activity and the detailed chemical constituents of red propolis from Shandong, China.

In this study, we prepared ethanol extracts of red propolis (EEP) from Shandong, China, evaluated its antioxidant activity, and identified the individual phenolic constituents in EEP. The antioxidant activity was compared with that of propolis from Brazil (red and green), Cuba (red) and Henan, China (poplar origin). We used four antioxidant assay systems: 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization, ferric ion reducing antioxidant (FRAP) and oxygen radical absorbance capacity (H-ORAC) assays. Furthermore, we quantitatively analyzed the major constituents of EEP using HPLC with photodiode array (PDA) detection.

Materials and Methods

Materials Propolis samples were supplied by Api Corporation as crude materials (Brazil and China) or ethanol extracts (Cuba). The origin of Brazilian green propolis is Baccharis dracunculifolia (Kumazawa et al., 2003). The crude propolis samples (100 mg each) were extracted with ethanol (3 mL) at room temperature for 24 h. The ethanol suspensions were separated by centrifugation at 1,000 rpm for 5 min at 25°C, and each supernatant was concentrated under reduced pressure to give EEP.

Caffeic acid (ε), cinnamic acid (ε), p-coumaric acid (g), ferulic acid (i), fluorescein, 2,4,6-tripryridyl-1,3,5-triazine (TPTZ), α-tocopherol (VE) and Trolox (6-hydroxy-2,5,7,8-tetramethylethrom-2-carboxylic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Apigenin (a), chrysin (d), galangin (j), pinocembrin (o), quercetin and tectochrysin (q) were purchased from Funakoshi (Tokyo, Japan). Folin-Ciocalteu reagent, aluminum chloride, butylated hydroxytoluene (BHT) and potassium persulfate were purchased from Kanto Chemicals (Tokyo, Japan). ABTS, 2,2′-azobis(2-aminopropene)dihydrochloride (AAPH), DPPH, gallic acid and phenethyl caffeate (caffeic acid phenethyl ester) were purchased from Wako Pure Chemicals Industries (Osaka, Japan). Benzyl caffeate (b), 3,4-dimethoxyxycinnamic acid (h), pinobanksin (l), pinobanksin 3-acetate (m), pinobanksin 5-methyl ether (n) and pinostrerin (p) was isolated from the ethanol extract of Uruguayan propolis (Kumazawa et al., 2002).

Total polyphenol and flavonoid content The total polyphenol content of EEP samples was determined by the Folin-Ciocalteu colorimetric method, with slight modifications (Singleton et al., 1999). EEP solution (0.5 mL) was mixed with 0.5 mL of 10% Folin-Ciocalteu reagent. After 3 min, 0.5 mL of 10% Na2CO3 was added, and the absorbance at 760 nm was measured after 1 h incubation at room temperature. EEP samples were evaluated at a final concentration of 20 μg/mL. Total polyphenol content was calculated as gallic acid equivalents (mg/g of EEP) from a calibration curve.

The flavonoid content of EEP samples was determined by a colorimetric method using aluminum chloride (Woisky and Salatino, 1998). To 0.5 mL of EEP solution was added 0.5 mL of 2% aluminum chloride-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 content was calculated as quercetin equivalents (mg/g of EEP) from a calibration curve.

DPPH radical scavenging activity The effect of DPPH radical-scavenging was evaluated according to the method of Chen and Ho (1995), with some modifications. To 1.25 mL of EEP solution (24 μg/mL) was added 0.25 mL of 0.5 mM DPPH solution. After 1 h incubation at room temperature, the absorbance was read at 517 nm. Results were expressed as percent decrease with respect to control values. EEP samples were evaluated at a final concentration of 20 μg/mL, with BHT and VE at the same concentration used as reference samples.

ABTS radical scavenging activity ABTS radical cation (ABTS·+) scavenging activity was measured according to the method described by Erel (2004), with some modifications. ABTS was dissolved in water to a final concentration of 7 mM. The ABTS radical cation was produced by reacting the ABTS stock solution with 2.45 mM potassium persulfate (final concentration) in the dark at room temperature for 12−16 h to allow completion of the radical generation reaction. This solution was then diluted twentyfold with ethanol. To determine the scavenging activity of an EEP sample, 180 μL of diluted ABTS·+ solution was added to each well of a clear-bottom, 96-well microplate. EEP solution (20 μL, 1 mg/mL)
was added to each well, and the absorbance was measured at 734 nm 5 min after initial mixing, using ethanol as the blank. The percent inhibition was calculated by the equation:

\[
\% \text{ inhibition} = 100 \times \frac{(A_c - A_s)}{A_c}
\]

where \(A_c\) is the absorbance of the control and \(A_s\) is the absorbance of the sample. Trolox, BHT and VE were prepared as positive control samples. All EEP samples and controls were evaluated at a final concentration of 0.1 mg/mL.

**Ferric reducing activity power (FRAP) assay** The FRAP assay was carried out as described by Benzie and Strain (1999), with slight modifications. Briefly, FRAP reagent consisted of 10 mM TPTZ solution in 40 mM hydrochloric acid, 300 mM sodium acetate buffer (pH 3.6) and 20 mM ferric chloride (III) solution in the ratio 10:1:1 (v/v/v), respectively. A volume of 0.1 mL of EEP solution (0.5 mg/mL) was added to 3 mL of FRAP reagent and incubated at room temperature for 3 min, then the absorbance at 593 nm was measured immediately. The results were calculated as \(\mu\)g ascorbic acid equivalent/mL.

**H-ORAC FL assay** The H-ORAC FL assay was carried out as described by Prior et al. (2003), with minor modifications. Fluorescein and AAPH were dissolved in 75 mM phosphate buffer, pH 7.4. A 25 \(\mu\)L aliquot of blank, Trolox standard or EEP in ethanol was added to quadruplet wells of a clear-bottom, 96-well microplate. Then, 150 \(\mu\)L of 8.38 nM fluorescein was added to each well, the plate was incubated at 39°C for 10 min, and 25 \(\mu\)L of prepared 153 mM AAPH was added to each well using an 8-channel pipette. Fluorescence decay was monitored at 528 nm upon excitation at 485 nm for 1.5 h. The area under the fluorescence versus time curve for each sample minus the area under the curve for the blank was calculated; these areas were compared to a standard curve prepared from the area under the curve for 3.13, 6.25, 12.5, 25, 50 and 100 \(\mu\)M Trolox standards minus the area under the curve for the blank. H-ORAC FL values were expressed as \(\mu\)mol of Trolox equivalents (TE)/g of EEP.

**HPLC analysis with PDA** HPLC with PDA detection was used to identify and determine the constituents in EEP. EEP samples were diluted with ethanol (2 mg/mL) and filtered through a 0.45 \(\mu\)m filter (German Sciences, Tokyo, Japan) prior to the injection of a 10 \(\mu\)L aliquot into the HPLC column. HPLC analysis was performed using a Jasco HPLC system (Tokyo, Japan) equipped with a reversed phase Capcell Pak C18 UG120 column (250 × 4.6 mm i.d., 5 \(\mu\)m; Shiseido, Tokyo, Japan) or Develosil C30 RPAQUEOUS column (250 × 4.6 mm i.d., 5 \(\mu\)m; Nomura Chemical, Aichi, Japan). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B). The gradient was 20 − 100% B (0 − 80 min) at a flow rate of 1.0 mL/min. For analysis by PDA detection, UV spectra were recorded from 195 to 650 nm at a rate of 0.8 spectra/s and with a resolution of 4.0 nm.

**Results and Discussion**

**Total polyphenol and flavonoid content of propolis samples** It has been suggested that flavonoids and other phenolic substances play a preventive role in the development of cancer and heart disease (Pandey and Rizvi, 2009). The Folin-Ciocalteu and AlCl3 coloration methods are widely used to determine total polyphenol and flavonoid content, respectively (Siatka and Kašparová, 2010). Here, we applied these methods to determine the total polyphenol and flavonoid content of red propolis from Shandong, China. These physicochemical methods are useful for evaluating various propolis samples because propolis contains many kinds of phenolics.

Table 1 shows the collection site, color, and total polyphenol and flavonoid content of the propolis samples used in the present study. The color of propolis from Shandong (1) was pale red. As shown in Table 1, the total polyphenol content in EEP of red propolis from Shandong was very high, 433.8 ± 1.7 mg/g of EEP. Propolis from Alagoas, Brazil (2) had the next highest polyphenol content, 292.7 ± 2.9 mg/g of EEP. We previously determined the polyphenol content of propolis from various geographical locations (Ahn et al., 2004; Ahn et al., 2007; Hamasaka et al., 2004; Kumazawa et al., 2004b). The polyphenol content of EEP from Japan and Korea was 100 − 200 and 150 − 250 mg/g of EEP, respectively (Ahn et al., 2004; Hamasaka et al., 2004). Thus, red propolis from Shandong, China has a significantly higher polyphenol content.

<table>
<thead>
<tr>
<th>Propolis Collection site</th>
<th>Color</th>
<th>Total polyphenol (mg/g of EEP)</th>
<th>Flavonoids (mg/g of EEP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Shandong, China</td>
<td>Pale red</td>
<td>433.8 ± 1.7</td>
<td>129.6 ± 1.1</td>
</tr>
<tr>
<td>2 Alagoas, Brazil</td>
<td>Red</td>
<td>292.7 ± 2.9</td>
<td>140.2 ± 2.1</td>
</tr>
<tr>
<td>3 Cuba</td>
<td>Red</td>
<td>127.3 ± 2.7</td>
<td>25.7 ± 1.2</td>
</tr>
<tr>
<td>4 Henan, China</td>
<td>Dark brown</td>
<td>215.3 ± 1.6</td>
<td>54.7 ± 1.3</td>
</tr>
<tr>
<td>5 Minas Gerais, Brazil</td>
<td>Dark green</td>
<td>120.0 ± 5.6</td>
<td>51.9 ± 2.4</td>
</tr>
</tbody>
</table>

Values indicate the mean ± standard deviation.
The DPPH free radical scavenging activity of various EEP samples is shown in Fig. 1. The DPPH free radical scavenging activity assay system is a simple method for evaluating the antioxidant activity of compounds. We evaluated various EEP samples and reference compounds (BHT and VE) at a final concentration of 20 μg/mL. As shown in Fig. 1, EEP of propolis from Shandong, China (1) and Alagoas, Brazil (2) had strong DPPH radical scavenging activities of 98.8 ± 1.0% and 98.1 ± 1.0%, respectively. EEP from Cuba (3) and Henan, China (4) also had high activity (81.1 ± 1.1% and 70.5 ± 1.2%, respectively), but EEP from Minas Gerais, Brazil (5) showed low activity (52.8 ± 1.3%).

Previously, we reported that the DPPH free radical scavenging activity of Brazilian (Minas Gerais) propolis was weaker than that of Chinese propolis (Kumazawa et al., 2004b). The present studies confirmed these findings. Brazilian propolis has been classified into several groups, and the components of these classified propolis were also altered (Park et al., 2002). Thus, the DPPH free radical scavenging activity of propolis from Alagoas and Minas Gerais, Brazil is considered to be different.

Effects of various propolis samples on ABTS radical cation

Figure 2 shows the ABTS radical cation scavenging activity of various EEP samples. The ABTS radical cation scavenging assay is a spectrophotometric method widely used for assessing the antioxidant activity of various substances (Xu and Chen, 2011). The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate and is reduced in the presence of hydrogen-donating antioxidants. We evaluated various EEP samples and reference compounds (BHT and VE) at a final concentration of 1 mg/mL. As shown in Fig. 2, EEP from Shandong, China (1) had the strongest ABTS radical cation scavenging activity, 90.9 ± 0.6%. EEP from Henan, China (4) and Alagoas, Brazil (2) had the next highest activities of 84.0 ± 3.1% and 83.2 ± 1.7%, respectively. In contrast, EEP from Cuba (3) showed a relatively weak ABTS radical cation scavenging activity of 54.2 ± 3.0%.

The ABTS radical scavenging activity shown in Fig. 2 appears to correlate with the total polyphenol content, since propolis samples with high polyphenol contents, shown in Table 1, have high ABTS radical scavenging activity. However, more detailed qualitative and quantitative analyses of the active compounds will be necessary in order to elucidate the radical scavenging activity of propolis.

Ferric reducing activity power (FRAP) assay

FRAP activity of various EEP samples is shown in Fig. 3. The principle behind this method is the reduction of a ferric-tripyridyl-triazine complex to its ferrous form. We evaluated various EEP and reference compounds (BHT and VE) at a final concentration of 0.5 mg/mL. As shown in Fig. 3, EEP from Shandong, China (1) had the strongest FRAP activity (89.2 ± 3.8 μg/mL), whereas the FRAP activity of EEP from Alagoas, Brazil (2), Minas Gerais, Brazil (5), Henan, China (4), and Cuba (3) were 45.4 ± 2.4 μg/mL, 40.7 ± 2.1 μg/mL, 34.5 ± 1.1 μg/mL and 32.1 ± 0.5 μg/mL, respectively.

Although red propolis from Shandong, China showed strong antioxidant activity in DPPH and ABTS assays, it had particularly potent antioxidant activity in the FRAP assay.
As described above, the FRAP assay is based on the reduction of a ferric cation. These results suggest that red propolis from Shandong, China contains large amounts of compounds capable of reducing ferric cations.

**H-ORAC FL assay**  The H-ORAC FL values of various EEP samples are shown in Fig. 4. These values represent the scavenging activity against peroxyl radical induced by AAPH (Prior et al., 2003). The ORAC FL assay is designed to measure the antioxidant activity of foods against peroxyl radical. A wide variety of foods have been tested using this method; therefore, ORAC is a standardized method for measuring the antioxidant potency of foods (Niki, 2010). As shown in Fig. 4, EEP from Shandong, China (1) showed the highest ORAC value of 14900 ± 443 μmol TE/g of EEP. The H-ORAC FL values of EEP samples obtained from Minas Gerais, Brazil (5), Alagoas, Brazil (2), and Henan, China (4) were 8240 ± 658 μmol TE/g of EEP, 7740 ± 623 μmol TE/g of EEP, and 6380 ± 607 μmol TE/g of EEP, respectively. EEP from Cuba (3) had the lowest ORAC value of 4090 ± 67 μmol TE/g of EEP.

Although all EEP samples used in this study had high ORAC values, red propolis from Shandong, China showed the highest ORAC value. In all antioxidant assays, red propolis from Shandong, China showed remarkably higher activity than the other propolis samples. Thus, red propolis from Shandong, China could be a potent antioxidant food material.

**HPLC analysis of red propolis from Shandong, China**

We identified the major components in the EEP sample from Shandong, China by HPLC analysis with PDA detection (Table 2). To identify each peak, UV spectra of all components were measured. As shown in Table 2, the major components in the EEP sample from Shandong, China were Apigenin, Benzyl caffeate, Caffeic acid, Chrysin, Cinnamic acid, and 3,4-Dimethoxy cinnamic acid.

Table 2. Retention time ($t_R$) value, UV $\lambda_{max}$ and contents of the constituents in EEP from Shandong, China.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$t_R$ (min)</th>
<th>$\lambda_{max}$ (nm)$^b$</th>
<th>Contents (mg/g of EEP)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apigenin (a)</td>
<td>20.2</td>
<td>268, 337</td>
<td>15.4 ± 0.8</td>
</tr>
<tr>
<td>Benzyl caffeate (b)</td>
<td>29.3</td>
<td>245, 331</td>
<td>21.1 ± 2.1</td>
</tr>
<tr>
<td>Caffeic acid (e)</td>
<td>5.7</td>
<td>291, 319</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>Chrysin (d)</td>
<td>29.6$^a$</td>
<td>270, 314</td>
<td>47.2 ± 3.7</td>
</tr>
<tr>
<td>Cinnamic acid (e)</td>
<td>8.8</td>
<td>290, 322</td>
<td>4.2 ± 0.6</td>
</tr>
<tr>
<td>Cinnamyl caffeate (f)</td>
<td>34.0</td>
<td>249, 330</td>
<td>7.6 ± 0.6</td>
</tr>
<tr>
<td>p-Coumaric acid (g)</td>
<td>8.0</td>
<td>291, 307</td>
<td>6.8 ± 0.7</td>
</tr>
<tr>
<td>3,4-Dimethoxy cinnamic acid (h)</td>
<td>13.6</td>
<td>291, 318</td>
<td>18.8 ± 1.2</td>
</tr>
<tr>
<td>Ferulic acid (i)</td>
<td>9.3</td>
<td>292, 320</td>
<td>9.8 ± 0.5</td>
</tr>
<tr>
<td>Galangin (j)</td>
<td>31.0</td>
<td>258, 361</td>
<td>101.6 ± 4.5</td>
</tr>
<tr>
<td>Phenethyl caffeate (k)</td>
<td>31.6$^a$</td>
<td>244, 330</td>
<td>32.7 ± 2.3</td>
</tr>
<tr>
<td>Pinobanksin (l)</td>
<td>24.5</td>
<td>292</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>Pinobanksin 3-acetate (m)</td>
<td>31.3$^a$</td>
<td>293</td>
<td>85.7 ± 3.4</td>
</tr>
<tr>
<td>Pinobanksin 5-methyl ether (n)</td>
<td>16.7</td>
<td>288</td>
<td>17.2 ± 1.1</td>
</tr>
<tr>
<td>Pinocembrin (o)</td>
<td>30.4$^a$</td>
<td>293</td>
<td>38.2 ± 2.8</td>
</tr>
<tr>
<td>Pinostrobin (p)</td>
<td>40.2</td>
<td>287</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>Tectochrysin (q)</td>
<td>40.8</td>
<td>271, 312</td>
<td>10.6 ± 1.1</td>
</tr>
</tbody>
</table>

$^a$ Separated by a Develosil C30 RPAQUEOUS column.
$^b$ UV values presented are from the HPLC chromatogram of a well-separated mixture of the standard.
$^c$ Values indicate the mean ± standard deviation.
peaks were compared with those of authentic standards. The chemical structures of the compounds identified are shown in Fig. 5. We identified 17 compounds: 9 flavonoids, 5 aromatic carboxylic acids, and 3 phenolic acid esters. All of these compounds have been reported to be identified from various propolis (Ahn et al., 2004; Ahn et al., 2007; Bankova et al., 1992; Hamasaka et al., 2004; Kumazawa et al., 2002; Kumazawa et al., 2004b; Kumazawa et al., 2010; Velikova et al., 2001).

The results of the quantitative analysis of EEP from Shandong, China are shown in Table 2. Values are expressed as means of triplicate analyses. The characteristic compounds

![Chemical structures of compounds](image)

Next, we quantitatively analyzed each component from the calibration curve of the HPLC chromatogram using the standards. However, portions of peaks d, k, m and o overlapped on the chromatogram obtained using the ODS column. These peaks were completely separated and quantified using C30 column (Kumazawa et al., 2010).

The results of the quantitative analysis of EEP from Shandong, China are shown in Table 2. Values are expressed as means of triplicate analyses. The characteristic compounds

![Chemical structures of compounds](image)

Fig. 5. Structures of the compounds in EEP of red propolis from Shandong, China.
of poplar-derived propolis are chrysin (d), pinocembrin (o), galangin (j) and tectochrysin (q) (Fujimoto et al., 2001). These compounds were detected from EEP from Shandong, China, suggesting that the main plant source of this propolis is poplar.

EEP from Shandong, China showed strong antioxidant activity, as described above. As shown in Table 2, EEP from Shandong, China had large amounts of the antioxidant compounds, galangin (j) and phenethyl caffeate (k): 101.6 and 32.7 mg/g of EEP, respectively. Previously we analyzed the phenolics in propolis from various areas of China, and detected galangin and phenethyl caffeate in most Chinese propolis (Ahn et al., 2007). Further, the content of galangin and phenethyl caffeate in these Chinese propolis was 3.8 – 12.8 and 0.6 – 8.7 mg/g of EEP, respectively (Ahn et al., 2007). Compared with these previous results, red propolis from Shandong, China contains a large amount of galangin and phenethyl caffeate. Galangin and phenethyl caffeate have been reported to have high antioxidant activity (Kumazawa et al., 2004b). Thus, the high antioxidant activity of red propolis from Shandong, China, may be ascribable to these compounds.

In this study, we investigated the in vitro antioxidant activity of red propolis from Shandong, China. Furthermore, the major polyphenols in this propolis were identified and quantitatively analyzed. Red propolis from Shandong, China has a large amount of antioxidant compounds such as galangin and phenethyl caffeate. The red pigment of this propolis is unknown, and so further studies on the pigment and the biological activities of this propolis are in progress.

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