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Chemical Compositions and Antioxidant Activities of Water Extracts of Chinese Propolis

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ABSTRACT: The present study investigated the chemical composition and antioxidant activity of the water extract of propolis (WEP) collected from 26 locations in China. Spectrophotometry was used to determine the physicochemical properties and the chemical constituents of WEP. Phenolic compounds in WEP were identified by RP-HPLC-DAD with reference standards. The antioxidant activities [characterized by reducing power and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability] of WEP were also measured. Results show that epicatechin, *p*-coumaric acid, morin, 3,4-dimethoxycinnamic acid, naringenin, ferulic acid, cinnamic acid, pinocembrin, and chrysin are the major functional phenolic compounds in Chinese WEPs. Furthermore, most WEPs show strong antioxidant activities, which are significantly correlated with $E^{1\%}_{1cm}$, an index for the estimation of the quality of WEP. WEPs also contain many more active constituents than ethanol extracts of propolis.

KEYWORDS: propolis, water extract, chemical composition, RP-HPLC-DAD, antioxidant activity

INTRODUCTION

Propolis is a resinous and adhesive natural substance collected by honeybees (*Apis mellifera* L.) from buds and leaves of plants, normally mixed with pollen as well as enzymes secreted by bees.[1] Propolis has been used in folk medicine for many years, especially in Europe and Asia. Its physical appearance (e.g., color, texture, and scent) varies widely and is determined by many factors, including mainly its complex chemical composition. Raw propolis generally consists of 50% resin (flavonoid and related phenolic acids, known as the polyphenolic fraction), 30% wax, 10% essential oil, 5% pollen, and 5% other organic compounds.[2] To date, more than 300 different constituents have been identified in propolis.[1,3,4] Evidence shows that propolis possesses anti-inflammatory, antibacterial, antiviral, immunomodulatory, antioxidant, and antiproliferative properties, attributed to the presence of flavonoids, phenolic acids, and its esters.[5−8]

Because lipophilic compounds are generally extracted conveniently by ethanol, applications and studies of propolis have focused mainly on the ethanol extract of propolis (EEP), whereas studies on the water extract of propolis (WEP) are lacking, except for those in Brazilian green propolis. WEP normally contains a mixture of natural substances, such as amino acids, phenolic acids, phenolic acid esters, flavonoids, cinnamic acid, and caffeic acid, whereas Brazilian green propolis is rich in terpenoids and prenylated derivatives of *p*-coumaric acids [9] and contains more hydrophilic constituents than other propolises. These compounds in Brazilian green propolis can be extracted efficiently by water.[10] Many chemical constituents have been isolated from the WEP of Brazilian propolis, including cinnamic acid and its derivatives (*p*-coumaric acid, artepillin C, drupanin, isosakuranetin, baccharin), caffeoylquinic acid derivatives (dicaffeoylquinic acid), 3-mono-*O*-caffeoylquinic acid (chlorogenic acid), caffeic acid, and flavonoids.[11−18] A study has shown that the WEPs of six Brazilian and one Chinese propolis possessed stronger 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability than their methanol extracts.[19] The major constituents (including caffeoylquinic acids) in the WEP of Brazilian green propolis have greater antioxidant effects, greater inhibitory activity against some enzymes, and better absorbency than its EEP.[14] WEPs are also known to have hepatoprotective activity in both chemical and immunological liver injury models,[20] antiviral activity, inhibition of platelet aggregation,[21] bacteriostasis,[22] and antiinflammatory activity.[23] Because WEP can prevent the side effects caused by alcohol in EEP and is absorbed easily by animals,[24] it is worth further studying of the chemical composition and antioxidant activities of WEP.

The chemical constituents of propolis vary greatly depending on various factors, such as plant resources, collecting seasons, species of bees, and the solvents used in extraction.[4,12,18,25−28] Our previous study on the chemical compositions and antioxidant activities of EEP showed remarkable differences among 29 propolis samples collected from 20 provinces in China.[29] The purposes of the present study are to characterize the physicochemical properties and major chemical constituents and to measure the antioxidant activities of WEP from different areas in China. Moreover, the relationships between chemical compositions and antioxidant activities of Chinese WEPs are also discussed.

MATERIALS AND METHODS

Propolis Samples. Twenty-six crude propolis samples (13 from temperate zones, 12 from subtropical zones, and 1 from a tropical zone) were obtained from various locations in China (Figure 1).

Chemicals. Cinnamic acid, quercetin, naringenin, genistein, kaempferol, apigenin, *p*-coumaric acid, and ferulic acid were purchased from the National Pharmaceutical Engineering Center for Solid Preparation

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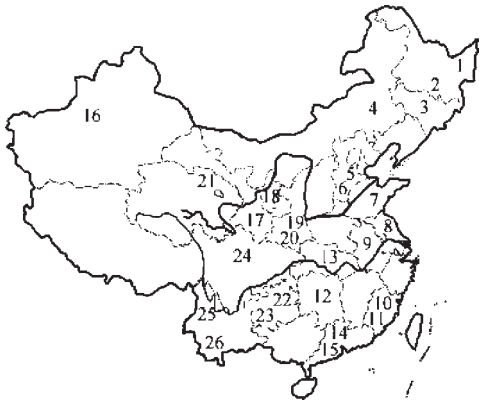

Figure 1. Provenance of 26 propolis samples in China. Propolis samples from temperate zone: 1, HLJ-1, Heilongjiang Province (Mudanjiang); 2, HLJ-2, Heilongjiang Province (Raohe); 3, JL, Jilin Province (Jilin); 4, NM, Neimenggu Province (Wulanhaote); 5, HeB-1, Hebei Province (Cangzhou); 6, HeB-2, Hebei Province (Shijiazhuang); 7, SD, Shandong Province (Jining); 8, JS, Jiangsu Province (Nanjing); 16, XJ, Xinjiang Province (Yili); 17, GS, Gansu Province (Tianshui); 18, NX, Ningxia Province (Yanchi); 19, SX-1, Shanxi Province (Xi'an); 20, SX-2, Shanxi Province. Propolis samples from subtropical zone: 9, AH, Anhui Province (Huangshan); 10, FJ-1, Fujian Province (Fuzhou); 11, FJ-2, Fujian Province (Fuzhou); 12, HuN, Hunan Province (Yongzhou); 13, HuB, Hubei Province (Suizhou); 14, GD-1, Guangdong Province (Lechang); 15, GD-2, Guangdong Province (Maoming); 21, QH, Qinghai Province (Huzhu); 22, GZ-1, Guizhou Province (Zunyi-Loudi); 23, GZ-2, Guizhou Province (Zunyi-Shibanqiao); 24, SC, Sichuan Province (Longchang); 25, YN-1, Yunnan Province (Lijiang). Propolis samples from tropical zone: 26, YN-2, Yunnan Province (Xishuangbanna).

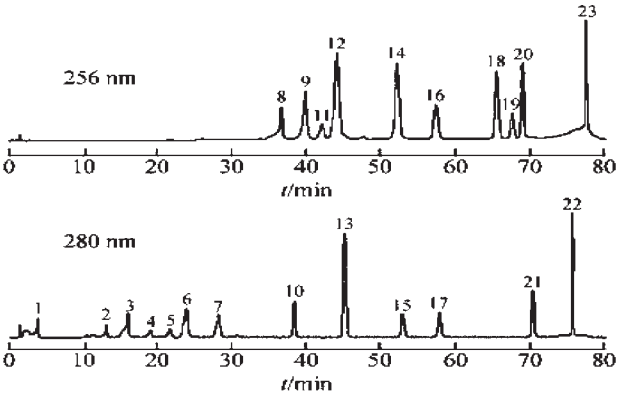

Figure 2. Chromatograms of mixed 23 reference standards after grouping at 256 and 280 nm. Peaks: 1, gallic acid (3.82); 2, catechin (13.70); 3, epicatechin (16.22); 4, caffeic acid (19.32); 5, α-catechin (21.13); 6, *p*-coumaric acid (24.32); 7, ferulic acid (28.55); 8, rutin (36.77); 9, myricetin (40.35); 10, 3,4-dimethoxycinnamic acid (38.85); 11, fisetin (42.24); 12, morin (44.21); 13, cinnamic acid (45.70); 14, quercetin (52.38); 15, naringenin (52.92); 16, luteolin (58.06); 17, genistein (58.16); 18, kaempferol (65.73); 19, apigenin (67.11); 20, isorhamnetin (68.35); 21, baicalin (69.13); 22, pinocembrin (76.00); 23, chrysin (76.48). The number in parentheses is the retention time of the chemical; the measurement unit is minutes.

in Chinese Herbal Medicine (Jiangxi, China). Gallic acid, catechin, epicatechin, caffeic acid, luteolin, isorhamnetin, morin, baicalin, α-catechin, rutin, 3,4-dimethoxycinnamic acid, and chrysin were purchased from the National Institute for the Control of Pharmaceutical and Biological Product (Beijing, China). Myricetin, fisetin, pinocembrin, and DPPH were purchased from Sigma-Aldrich Chemicals Co., Ltd. (St. Louis, MO). Methanol and formic acid were bought from Merck (Darmstadt, Germany). Other reagents were of analytical grade.

Preparation of Water Extract of Propolis. Propolis samples were frozen at −18 °C and ground into powder by a mill. Four grams of powder was dissolved in 20 mL of distilled water at 60 °C for 7 h. The crude extract was filtered, and the residue was re-extracted under the same conditions. Both extracts were centrifuged at 28000*g* for 30 min, and the supernatants were concentrated under reduced pressure to produce the WEP. The average WEP yield of 26 propolis samples was 6.0 ± 1.6%. The WEP solution (10 mg/mL H_2O) was used as the sample solution for further experiments.

Specific Absorbance of UV Spectrum. The UV absorption spectra of each WEP and its maximum absorption (λ_{max}) were measured in the 400−190 nm region with a Shimadzu UV-1700 spectrophotometer. The WEPs were diluted using distilled water in a 25 mL volumetric flask to a final concentration of 25 or 50 μg/mL. Most of the WEP solutions were prepared so that the absorbance ranged from 0.5 to 1.0. The spectra were normalized for the fraction of sample dissolved in water and reported as specific absorption $E^{1\%}_{1cm}$, the absorbance of a 10000 μg/mL solution.

Total Polyphenol Contents. Total polyphenol contents in WEP were determined by the Folin−Ciocalteu colorimetric method according to the method of Tawaha et al.[30] Briefly, 20 μL of WEP (10 mg/mL) was mixed with 3.0 mL of the Folin−Ciocalteu reagent and 2.0 mL of 20% Na_2CO_3 and then agitated and diluted to 25 mL using distilled water. The absorbance was measured at 765 nm after 1.5 h of incubation at 30 °C with intermittent shaking. Total polyphenol contents were expressed as gallic acid equivalent (mg/g).

Flavone−Flavonol and Flavanone Contents. The flavone−flavonol and flavanone contents were measured using the method of Ivan et al.[31] with minor modifications. To determine the flavone−flavonol content, 1.0 mL of WEP (10 mg/mL) was mixed with 3.0 mL of 95% alcohol, then 2.5 mL of 10% $AlCl_3$ and 2.5 mL of 1 mol/L KAc were added, and the mixture was agitated and then diluted to 25 mL using distilled water. After 15 min at room temperature, the absorbance was measured at 415 nm. Flavone−flavonol contents were calculated as quercetin equivalent (mg/g).

To determine the content of flavanone, 1.0 mL of WEP (10 mg/mL) was mixed with 2.0 mL of 1% DNPD−H_2SO_4 and 2.0 mL of 99.8% methanol and then kept in a water bath at 50 °C for 50 min. After cooling at room temperature, the solution was mixed with 5 mL of 1% KOH in distilled water. Then, 1 mL of the mixture was taken and centrifuged at 28000*g* for 10 min; the supernatants were adjusted to 25 mL. The absorbance of the supernatants was measured at 495 nm. Flavanone contents were calculated as naringenin equivalent (mg/g) from the calibration curve generated by plotting absorbance versus naringenin concentration (mg/mL).

Soluble Carbohydrate Contents. Ten microliters of WEP (10 mg/mL) was mixed with 0.9 mL of distilled water and kept in an ice bath for 5 min and then added into 4 mL of anthracenone solution (1 mg/mL), which was mixed with 80% sulfuric acid. After the mixtures were boiled in a water bath for 10 min and then cooled at room temperature, the absorbance was measured at 620 nm. Soluble carbohydrate contents were expressed as glucose equivalent (mg/g).

RP-HPLC Analysis of WEP. To analyze the chemical compounds in WEP, 23 polyphenolic chemicals were chosen as reference standards. After filtration using a Millex-LH filter (0.45 μm, Agilent, Santa Clara, CA), 20 μL of WEP (10 mg/mL) was injected into the HPLC system equipped with an Agilent 1200 (USA) Zorbax Eclipse XDB C_{18} column (4.6 mm × 150 mm, 5 μm). The mobile phase contained methanol (A) and 0.1% formic acid in water (B). The detection wavelengths of the diode array detector (DAD) were set as 256 and 280 nm. The gradient

Table 1. $E^{1\%}_{1cm}$, Total Polyphenol, Flavanone, Flavone−Flavonol, and Soluble Carbohydrate Contents of Water Extract of Propolis from 26 Locations in China

sample	$E^{1\%}_{1cm}$ [a]	total polyphenol ($mg_{gallic\ acid}/g$)	RSD (%)	flavanone ($mg_{naringenin}/g$)	RSD (%)	flavone−flavonol ($mg_{quercetin}/g$)	RSD (%)	soluble carbohydrate ($mg_{glucose}/g$)	RSD (%)
HLJ-1	166.1 (289.5)	377.25	0.36	5.90	4.41	10.90	0.73	300.75	2.46
HLJ-2	164.2 (286.5)	172.95	0.29	4.96	3.83	9.44	1.91	240.75	4.20
JL	278.3 (291.5)	321.45	0.16	5.44	3.86	8.87	1.69	235.51	1.15
NM	200.5 (289.0)	358.35	0.29	6.51	3.69	14.04	0.50	477.92	2.40
HeB-1	195.7 (289.0)	217.05	0.24	5.91	3.21	7.92	2.27	466.97	4.33
HeB-2	161.2 (288.0)	321.34	0.16	6.75	2.81	10.91	0.55	422.20	1.91
SD	291.6 (290.0)	376.65	0.24	5.32	4.70	15.33	0.39	176.93	1.14
FJ-1	117.2 (289.0)	190.35	0.24	5.20	4.81	9.83	1.02	247.89	4.35
FJ-2	131.1 (289.5)	159.75	1.49	6.54	3.37	6.48	2.31	301.23	3.59
JS	240.8 (290.5)	322.05	0.43	6.15	3.58	15.42	0.19	315.52	0.85
AH	155.3 (288.0)	192.75	0.97	5.80	1.90	7.93	1.90	427.44	0.16
HuN	96.5 (288.5)	90.45	0.97	6.28	1.75	7.17	1.95	327.90	4.52
HuB	196.5 (289.5)	376.95	0.69	5.91	2.20	14.31	0.35	310.28	1.95
GD-1	45.8 (287.0)	40.35	1.31	5.37	0.93	4.73	2.54	428.87	5.02
GD-2	60.7 (287.5)	58.05	1.57	4.75	1.47	5.39	2.23	225.03	2.40
SX-1	193.6 (289.0)	204.75	0.88	6.29	0.95	10.89	1.65	460.77	3.07
SX-2	116.6 (287.5)	195.75	0.46	6.68	2.10	10.12	1.48	315.04	3.64
GS	144.6 (288.0)	198.45	0.45	6.17	3.24	8.66	1.04	461.25	2.34
QH	113.1 (288.0)	156.78	0.34	6.88	2.76	9.56	1.67	609.36	3.43
NX	141.6 (287.5)	159.15	0.65	5.84	3.42	8.86	1.58	540.78	1.62
XJ	162.0 (286.5)	150.15	0.35	5.90	0.68	9.83	1.52	250.27	1.88
SC	168.3 (288.0)	241.95	1.41	5.37	2.80	10.68	1.50	317.42	0.42
GZ-1	166.8 (288.5)	170.55	0.53	5.86	0.68	7.55	1.85	616.51	4.81
GZ-2	200.5 (287.5)	325.95	0.35	7.97	4.52	8.77	1.25	370.76	2.54
YN-1	91.0 (286.0)	88.95	2.54	4.61	2.39	3.47	3.46	915.60	0.29
YN-2	−	10.05	5.17	5.42	5.17	4.83	7.25	455.06	2.53
av		210.70	51.45	5.91	12.35	9.30	33.55	392.96	40.46
av of EEP [b]	−	265.09		52.54		79.68		−	

[a] The relative standard deviation (RSD) is given in parentheses (n = 3). [b] The data are from ref 29. −, not detected.

was 15% A (10 min), 17% A (20 min), 32% A (30 min), 42% A (60 min), 50% A (70 min), 65% A (75 min), and 25% A (80 min) at a flow rate of 1.0 mL/min. For the analysis of DAD, UV spectra were recorded from 200 to 400 nm at a rate of 0.8 spectrum/s and a spectral resolution of 4.0 nm.[32] The t_R (minutes) values of the standards are shown in Figure 2. Calibration curves were generated to estimate the content of the main compounds in samples. The correlation between the concentration and the peak area was assessed by the ordinary least-squares regression model. The compounds in WEP were determined by comparing the retention time and the UV absorbance spectra with reference standards.

Reducing Power Measurement. One milliliter of each WEP with various concentrations (25, 50, 100, 200, and 400 μg/mL) was added into 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferrocyanate [$K_3Fe(CN)_6$].[33] The mixtures were incubated at 50 °C for 20 min, then 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 2000g over 10 min. The supernatants were collected and mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance was recorded at 700 nm. Rutin with the same concentration was used as the reference sample. The increase in absorbance represented the increase in reducing power.

DPPH Scavenging Activity Measurement. A blank (0.1 mL of methanol) and 0.1 mL of each WEP (100, 200, 300, 400, 500, 600 μg/mL) were each added to 3.9 mL of DPPH methanol solution (41 μg/mL) separately.[34] After 90 min of incubation at room temperature in a dark room, the absorbance was recorded at 517 nm. Results were expressed as antioxidant activity index (AAI) and calculated as follows:

$$I\% = [(Abs_{blank} - Abs_{WEP})/Abs_0] \times 100$$

$$AAI = [final concentration of DPPH\ (\mu g/mL)]/IC_{50}\ (\mu g/mL)$$

The IC_{50} (concentration providing 50% inhibition) was calculated on a calibration curve by plotting the extract concentration and the corresponding scavenging effect.

Statistical Analysis. All assays were carried out in triplicates. The data were analyzed using ANOVA, and results were expressed as the mean ± RSD% by SPSS version 13.0.0 for Windows (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

General. Measurements of the specific absorbance ($E^{1\%}_{1cm}$) and the contents of total polyphenol, flavone−flavonol, flavanone, and soluble carbohydrate of the Chinese WEPs are given in Table 1. The $E^{1\%}_{1cm}$ value of UV absorption is one of the physicochemical parameters that are used to evaluate the quality of propolis.[10] It is believed that various pharmacological activities of propolis are attributed to phenolics, including flavonoids and caffeic acids. The $E^{1\%}_{1cm}$ values of most Chinese WEPs fall in the

Table 2. Content of Chemicals in Water Extract of Propolis from 26 Locations in China

	content[a] (mg/g of WEP)																						
sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
HLJ-1	1.25	2.35	43.66	33.94	5.11	77.71	10.03	0.52	0.52	9.91	4.97	2.43	0.79	–	10.44	–	–	–	–	–	0.45	2.14	0.69
HLJ-2	–	6.35	14.43	11.78	–	8.83	6.30	–	3.44	12.03	2.85	24.07	6.58	–	7.05	0.50	0.48	–	–	1.03	0.47	1.83	–
JL	–	2.65	31.82	9.93	–	71.61	9.65	0.87	5.30	3.66	14.22	1.12	0.85	0.56	8.92	0.51	0.79	0.59	5.57	–	0.40	0.80	–
NM	–	0.98	51.14	5.13	–	3.45	1.66	–	1.58	16.66	–	1.33	0.67	–	9.47	0.47	0.53	–	–	–	0.38	1.60	0.81
HeB-1	–	18.60	25.63	24.17	–	23.91	5.27	1.47	3.04	11.06	3.02	8.32	–	–	8.62	0.65	0.50	–	–	0.64	0.39	1.33	0.69
HeB-2	–	2.20	35.50	7.56	3.96	9.33	3.68	–	1.78	15.41	–	2.71	0.93	–	5.49	0.46	0.69	–	1.73	–	0.42	1.55	0.70
SD	–	–	17.40	2.04	–	3.42	0.83	–	0.73	3.54	0.49	1.36	–	0.05	2.13	–	0.13	–	–	–	–	0.22	0.10
FJ-1	–	–	21.20	7.74	1.09	5.49	3.63	–	2.56	12.07	–	5.08	1.51	0.79	7.31	–	–	–	–	–	0.41	0.82	1.53
FJ-2	–	7.23	8.55	10.41	–	7.49	3.48	2.05	1.41	1.48	–	9.57	–	–	1.73	–	–	–	–	–	0.42	0.96	–
JS	–	8.76	59.93	13.62	–	17.45	7.61	3.25	1.62	15.88	5.24	14.83	4.13	–	8.50	0.88	0.57	–	–	–	0.43	1.10	–
AH	–	5.78	23.68	12.24	–	10.21	3.44	–	2.28	13.14	1.61	4.92	1.50	–	7.66	0.57	–	0.88	–	0.59	0.48	1.29	–
HuN	–	2.73	7.62	–	1.45	6.37	2.73	–	3.67	2.72	–	4.52	1.39	0.72	12.30	1.32	–	–	–	–	0.47	4.73	–
HuB	–	7.44	42.48	8.73	–	11.67	4.09	2.63	3.54	12.36	2.80	11.50	–	0.83	6.06	2.46	0.71	–	–	–	0.43	0.70	1.90
GD-1	–	–	1.42	1.09	–	0.92	1.10	–	1.02	1.08	–	1.41	–	–	5.34	0.46	–	–	–	–	–	1.42	0.67
GD-2	–	2.14	4.53	–	1.39	0.53	0.92	–	–	6.49	–	5.65	1.73	–	5.32	–	–	–	2.28	0.77	0.39	1.04	–
SX-1	–	0.93	34.60	11.24	–	9.91	10.01	–	47.23	1.37	35.78	13.32	3.64	1.53	8.23	0.46	–	–	–	17.68	0.61	1.28	3.22
SX-2	8.65	17.72	9.73	4.99	–	3.26	2.95	–	2.74	4.98	1.88	1.95	0.72	–	6.02	–	0.57	0.79	–	0.68	–	1.48	0.60
GS	–	8.20	26.66	–	1.75	5.77	2.23	–	6.36	12.01	1.39	2.44	0.86	–	8.91	0.53	0.70	–	–	0.95	0.45	0.84	0.53
QH	–	4.09	20.80	1.91	–	1.68	4.89	–	2.50	6.29	0.96	0.22	–	1.53	6.36	1.53	0.64	–	–	0.69	0.40	1.00	–
NX	–	3.76	24.03	–	–	4.01	1.51	0.51	3.41	16.26	–	4.04	1.28	–	8.75	0.80	0.51	–	–	0.69	0.43	1.40	0.69
XJ	–	10.51	19.69	11.13	–	9.25	3.76	–	3.23	13.01	0.99	5.03	1.50	–	7.36	0.89	0.51	–	–	0.79	0.40	1.31	0.63
SC	0.55	24.43	30.57	14.35	–	8.51	6.69	–	4.97	8.45	2.50	6.54	1.94	–	5.98	1.70	0.66	–	–	0.75	0.39	1.26	0.87
GZ-1	–	12.83	26.46	8.24	–	8.16	2.64	0.48	1.05	7.38	–	26.72	7.34	–	2.97	1.23	0.55	1.79	–	0.68	0.54	1.41	–
GZ-2	0.57	16.41	38.21	3.50	–	13.15	6.05	–	9.35	7.86	–	38.31	10.43	–	3.36	0.54		1.79	–	0.00	0.87	0.81	–
YN-1	–	8.22	14.01	–	1.00	5.56	1.23	–	0.74	7.59	1.84	31.31	8.25	–	3.17	0.40	–	0.77	–	0.63	0.39	1.66	0.80
YN-2	–	–	11.13	–	–	0.46	0.86	–	–	1.10	–	1.04	–	–	0.62	–	–	–	–	–	–	2.58	3.60

[a] Values are expressed as the mean of triplicates analyzed for each sample. 1, gallic acid; 2, catechin; 3, epicatechin; 4, caffeic acid; 5, α-catechin; 6, *p*-coumaric acid; 7, ferulic acid; 8, rutin; 9, myricetin; 10, 3,4-dimethoxycinnamic acid; 11, fisetin; 12, morin; 13, cinnamic acid; 14, quercetin; 15, naringenin; 16, luteolin; 17, genistein; 18, kaempferol; 19, apigenin; 20, isorhamnetin; 21, baicalin; 22, pinocembrin; 23, chrysin; –, not detected.

range of 120–290 (Table 1). These values were similar to those reported from Chinese propolis, but higher than those from Brazilian propolis previously reported by Miyataka et al.[10] Furthermore, the $E^{1\%}_{1cm}$ values of WEP from a few subtropical samples (YN-1, HuN, GD-1, and GD-2) are smaller than those of the other WEP samples. Particularly, the $E^{1\%}_{1cm}$ value of WEP from the tropical sample (YN-2) was not detected with absorbance in the 400–190 nm region.

Total polyphenol contents of WEP from four subtropical samples (YN-1, HuN, GD-1, and GD-2) and the single tropical sample (YN-2) showed low values (Table 1). The average content of total polyphenol of WEP was 210.70 mg/g, higher than that of flavanone and flavone–flavonol but lower than that of soluble carbohydrate. For Chinese samples, the contents of total polyphenol, flavanone, and flavone–flavonol in WEP were lower than those in EEP.[29] However, the content of total polyphenol of most WEPs was similar to the ones reported.[35] Soluble carbohydrate and total polyphenol were the main chemical compositions in WEP of Chinese samples. Sample HLJ-1 has the highest content of total phenol in WEP, being almost 37 times higher than that of YN-2. The differences among the above-mentioned four constituents in WEP were all statistically significant ($P < 0.05$).

RP-HPLC Analysis of the Chemical Composition of WEPs. Twenty-three reference standards in WEPs were determined qualitatively and quantitatively by RP-HPLC (Table 2). Values are expressed as means of triplicates for each sample, and the relative standard deviations ranged from 1.28 to 4.79%. The type of constituents and contents of phenolic in WEPs varied among the samples. Seven chemicals (epicatechin, *p*-coumaric acid, morine, 3,4-dimethoxycinnamic acid, naringenin, ferulic acid, and pinocembrin) were present in all WEPs. HA high content of caffeic acid was detected in most WEPs except samples YN-1, YN-2, GD-2, HuN, GS, and NX. Catechin, myricetin, cinnamic acid, chrysin, and fisetin were found in many WEPs as well. Apigenin was detected in only three samples (HeB-2, JL, and GD-2). A low content of kaempferol was found in YN-1, JL, GZ-1, GZ-2, GD-2, and SX-2. The other standards were detected either only in a few WEPs or with contents lower than 1 mg/g. The WEP of JL contained 19 (of 23) chemicals except for α-catechin, gallic acid, isorhamnetin, and chrysin. Eighteen chemicals were detected only in WEP of SC, whereas 17 chemicals were detected in WEPs of many other samples (HLJ-1, HB-1, HB-2, HuB, SX-1, SX-2, GS, XJ, GZ-1, and YN-1). However, only eight chemicals were detected in YN-2. The rest of the WEPs each contained 11–16 chemicals. These results show that these samples contain many more different chemicals than

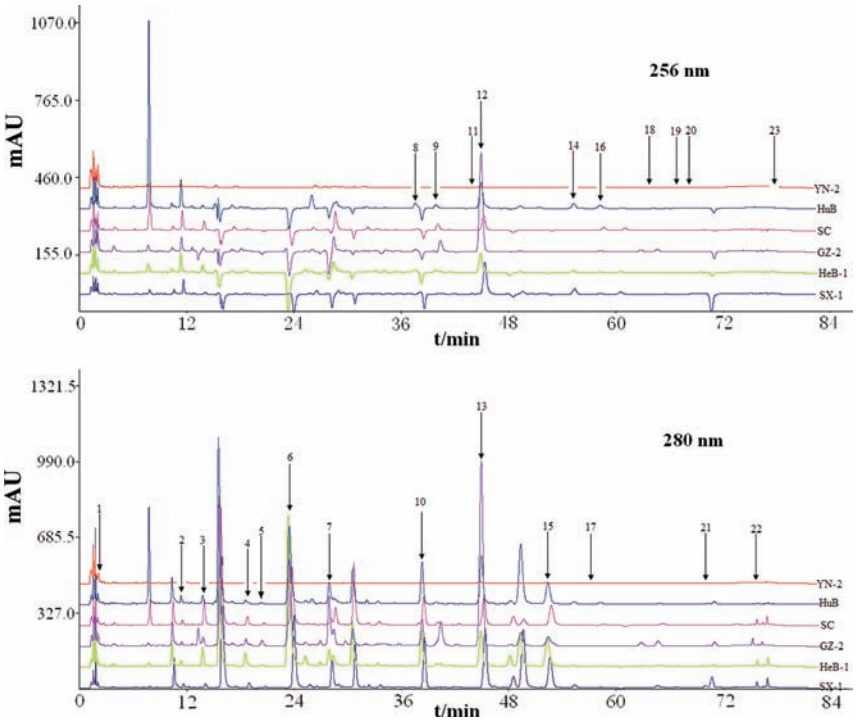

Figure 3. HPLC fingerprints at 256 and 280 nm of six WEPs representing different climatic zones. Peaks: 1. gallic acid; 2, catechin; 3, epicatechin; 4, caffeic acid; 5, α-catechin; 6, *p*-coumaric acid; 7, ferulic acid; 8, rutin; 9, myricetin; 10, 3,4-dimethoxycinnamic acid; 11, fisetin; 12, morin; 13, cinnamic acid; 14, quercetin; 15, naringenin; 16, luteolin; 17, genistein; 18, kaempferol; 19, apigenin; 20, isorhamnetin; 21, baicalin; 22, pinocembrin; 23, chrysin.

Brazilian WEP, which contained mainly 3,4-di-*O*-caffeoylquinic acid (6.1%), 3,5-di-*O*-caffeoylquinic acid (4.9%), *p*-coumaric acid (3.7%), and chlorogenic acid (3.6%).[16]

It is noted that HPLC fingerprints at 256 nm of five WEPs, except for sample YN-2, present negative peaks (Figure 3). The explanation for this phenomenon is that the absorptions of certain compounds, probably polyhydric alcohol compounds, glycosides or glycoside compounds, or salts in WEPs, were lower than that of the mobile phase under low wavelength detection. There were no negative peaks in the HPLC profiles of the reference standards, indicating negative peaks were not caused by HPLC equipment, solvents, mobile phases, pollution, or air bubbles (Figure 2). The compositions of WEPs were complex due to the lack of purification after extraction. For sample YN-2, which was collected from Xishuangbanna in Yunnan Province and contained rather simple components, no negative peaks were detected in its 256 nm profile. The appearance of negative peaks was relatively regular due to the similarity of the chemical composition of Chinese propolises (Figure 3). Because other major positive peaks were not interfered with by these negative peaks, the presence of these negative peaks did not change the interpretations of results in our study.

Antioxidant Activity of WEPs. The measurements of reducing power and DPPH scavenging activity of WEP and the ratio between WEP and EEP are given in Table 3. Because an increase in the slope rate (K) of the linear calibration curve, which is generated by plotting the concentrations versus the absorbance, was identical to an increase in reducing power,[33] the K value was used as an indicator for the reducing power of WEP. In the present study, the K values of most WEPs ranged from 1.2 to 3.47. The reducing power of WEP from the tropical sample (YN-2) and some subtropical samples (YN-1, HuN, GD-1, GD-2, and FJ-2) was demonstrated to be lower than that of other samples by having smaller K values. These small K values were similar to the ones reported for EEP,[29] suggesting a similar reducing power between WEP and EEP of Chinese samples.

DPPH scavenging activity was expressed as AAI, which varied from 0.28 (GD-1) to 3.29 (SD) (Table 3). According to Scherer's study, the sample would show poor, moderate, strong, or very strong antioxidant activities when AAI values were <0.5, between 0.5 and 1.0, between 1.0 and 2.0, or >2.0, respectively.[34] The AAI values of WEP from the tropical sample (YN-2) and subtropical samples of China (YN-1, GD-1, and GD-2) ranged from 0.28 to 0.77, indicating poor to moderate antioxidant activities in these WEPs. The other WEPs showed strong or very strong antioxidant activities. The AAI value of WEP was generally lower than that of corresponding EEP,[29] therefore indicating that the EEPs of Chinese samples had stronger DPPH scavenging activity than the WEPs.

The high reducing power and DPPH scavenging activities of WEP is most likely attributed to the total polyphenol. This is demonstrated by the significantly positive correlation (Table 4, $P < 0.01$). Moreover, the correlation coefficient between reducing power and AAI was also statistically significant, suggesting that the main constituents of WEP contributing to antioxidant activities are total polyphenol. Previous studies showed that there was a strong positive correlation between antioxidant activities and total phenol, and the content level of flavonoid largely influences the antioxidant activity of EEP.[36] Because $E^{1\%}_{1cm}$ had statistically significant correlations with total polyphenol, reducing power, and AAI, $E^{1\%}_{1cm}$ could be used as an indicator to estimate the quality of WEP.

This study clearly demonstrates that Chinese WEPs, especially those from temperate and some subtropical zones, are rich in

Table 3. Reducing Power and DPPH Scavenging Activity of WEP and the Ratio between WEP and EEP from 26 Locations in China

	WEP		WEP:EEP[a]	
sample	K^b	AAI[c]	K^b	AAI[c]
HLJ-1	2.27	2.48	0.92	0.63
HLJ-2	1.20	1.16	—	—
JL	1.61	1.42	1.59	0.56
NM	2.15	2.33	0.93	0.31
HeB-1	1.45	1.69	0.67	0.40
HeB-2	1.95	1.82	1.23	0.46
SD	3.47	3.29	2.03	0.85
FJ-1	1.54	1.31	0.73	0.33
FJ-2	0.78	0.75	0.54	0.19
JS	2.79	2.14	1.51	0.56
AH	1.43	1.31	0.65	0.34
HuN	0.63	0.58	0.60	0.23
HuB	2.23	2.10	—	—
GD-1	0.39	0.28	0.57	0.22
GD-2	0.53	0.52	0.77	1.13
SX-1	1.65	1.44	1.12	0.35
SX-2	1.50	1.73	1.09	0.45
GS	1.70	1.78	0.77	0.21
QH	1.44	1.39	0.61	0.14
NX	1.34	1.55	0.68	0.37
XJ	1.40	1.63	0.77	0.41
SC	1.72	2.07	0.86	0.25
GZ-1	1.65	2.09	0.78	0.26
GZ-2	2.32	2.23	1.00	0.52
YN-1	0.77	0.77	0.50	0.19
YN-2	0.20	0.46	0.45	0.59
rutin	4.22	6.36		

[a] The data of EEP are from ref 29. —, not detected. [b] Reducing power. [c] DPPH scavenging activity.

Table 4. Correlation between $E^{1\%}_{1cm}$, Chemical Compositions of Water Extracts of Propolis, and Their Antioxidant Activities

	$E^{1\%}_{1cm}$	total polyphenol[a]	reducing power[a]	AAI[a]
$E^{1\%}_{1cm}$	1.000	0.817**	0.817**	0.760**
total polyphenol		1.000	0.885**	0.853**
reducing power			1.000	0.954**
AAI				1.000

[a] Values significantly different by bivariate correlations test: **, $P < 0.01$.

bioactive components and have strong antioxidant activities. Some propolis samples are extracted more efficiently by water than by ethanol. Given these economically and medicinally favorable properties (e.g., WEP is relatively nontoxic, can be absorbed easily, and can prevent the side effects caused by alcohol in EEP), the Chinese WEPs may have a bright commercial prospect.

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