International Journal of Latest Engineering Research and Applications (IJLERA) ISSN: 2455-7137

Volume – 02, Issue – 06, June – 2017, PP – 36-47

Properties of Propolis from Different Areas of China

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Abstract: Fifty three propolis samples collected from different regions of China, one sample from Brazil and another from Madagascar were investigated. The balsam content extracted from crude propolis excellently correlated with bioactive components, including the total phenols (TP),total flavones/flavonols (TF) and total dihydro flavones / dihydro flavonols (TDF) content. DPPH radical (DPPHR) scavenging activity(SA) of Chinese propolis strongly correlated with theirTP content. The six minimum values, i.e., 1/EC50, balsam content, TP content, TF content and TDF content in propolis were given as the references of propolis quality standard.Cinnamic acidcontent correlated highly with naringenin content. The balsam content correlated highly with latitude that the balsam content decreases asthe latitude increases. The unexpected finding is that Apissinensis cannot produce propolis, but ApismelliferaL. can. orrelated with bioactive components, including the total phenols (TP),total

Keywords: Properties of propolis, Phenols, Flavonoids, DPPH radical scavenging, Apismellifera L., Apissinensis Chemical compounds studied in this article Caffeic acid (PubChem CID: 689043); p-Coumaric acid (PubChem CID: 637542); Ferulic acid(PubChem CID: 445858); Isoferulic (PubChem CID: 736186); 3,4-Dimethoxycinnamic acid (PubChem CID: 717531); Quercetin (PubChem CID: 5280343); Cinnamic acid (PubChem CID: 444539); Apigenin (PubChem CID: 5280443); Naringenin (PubChem CID: 932); Kaempferol (PubChem CID: 5280863); Chrysin (PubChem CID: 5281607); Pinocembrin (PubChem CID: 68071); Pinostrobin (PubChem CID: 73201)

1. Introduction

Propolis is a resinous, strongly adhesive natural substance, collected by honeybees (ApismelliferaL.) from buds and leaves of plants, mixed with pollen as well as enzymes secreted by bees[1]. In general, propolis is composed of about 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% other substances, including organic debris[1],[2]. Wax and organic debris are removed usually by 70% ethanol extraction, then propolisbalsam is obtained by removing solvent. The balsam contains over 300 bioactive constituents, including flavonoids, polyphenols, terpenoids, steroids, sugars and amino acids [1],[3],[4]. Propolis has been used in folk medicines in many regions of the world for thousands of years[5],[6]and is found to possess antiviral[4], anti-inflammatory[7] and anticancer[8]properties. For this reason, propolis is extensively used in food and beverages to improve health and prevent diseases such as inflammation, heart disease, diabetes and cancer [6],[9].

The antioxidant activities of propolis from various geographic origins were compared by Kumazawaet al.[6]. The ethanol extract of propolis (EEP) from Argentina, Australia, China, Hungary and New Zealand had relatively strong antioxidant activities and also correlated highly with the total phenols and flavonoids content.

In last decades, propolis and other beeproducts gained the interest of consumers and companies due to its high biological value proven through multiple effectson treatment of and also prevention from various diseases [10]. Although propoliswas intensively used in medicine, cosmetics and lately in food industry, there was no European quality standard for this specific beeproduct. There was a Chinese standard, but unfortunately its methods were outdated. In order to protect the consumers and the honest producers, it is urgent to establish a quality standard of propolis.

The composition of propolis depends on the vegetation of the collection site. Because of the geographical differences, propolis samples from Europe, South America and Asia have different chemical compositions[6]. The major components in propolis of Brazilian origin are terpenoids and prenylated derivatives of p-coumaricacids [6]. By contrast, propolis from Europe and China contains many kinds of flavonoids and phenolic acid esters[3],[6]. In temperate zones all over the world, poplar bud exudates (mainly of Populusnigra L.) have been shown to be the main source of propolis resin collected by bees. Undoubtedly, poplar-type propolis is studied most comprehensively and remains the best known type, both from chemical and pharmacological points of view[11],[12].

Fifteen Chinesepropolis samples were investigated and they significantly differed in their total phenols

and total flavonoids content, as well as their phytochemical profiles [13]. Additionally, the propolis samples differed in their DPPH, ABTS cation, hydroxyl and peroxide radicals scavenging activity and ferric reducing abilities[13]. Because there is giant variation of antioxidant activity and chemical composition of propolis different regions in China, it is difficult to establish the quality standard of Chinese propolis.

In our research, the analysis of 55 poplar-typepropolis samples from different geographic origins was reported. Among them, fifty three propolis samples were collected from different regions of China and one sample from Brazil and another from Madagascar in Africa separately.

2. Materials and methods

2.1. Materials

Analytical grade ethanol, methanol, quercetin, naringenin, gallic acid, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl radical(DPPHR), potassium hydroxide, aluminum nitrate, formic acid, potassium acetate, sodium carbonate, 2,4-dinitrophenylhydrazine (DNP), 96% sulfuric acid werepurchased from China Reagent Co. (Shanghai, China). Caffeic acid, pinocembrin, kaempferol, chrysin, naringenin, p-coumaric acid, ferulic acid, isoferulic acid, quercetin, apigenin, 3,4-dimethoxycinnamic acid, cinnamic acid and pinostrobin were purchased from Aladdin Industrial Corporation (Shanghai, China).HPLC grade acetonitrile were purchased from Sinopharm (Shanghai, China). Fifty-three propolis samples were collected from various locations in China (Fig. S1), including two others from Brazil and Madagascar in Africa separately (Table 1) and were stored under -25 °C before use.

2.2. Methods

1 Gram of the propolis was extracted with 60 mL 70% ethanol in a 100mL flask at room temperature for 8hours. The three extracts were combined and filtered under reduced pressure then diluted to 100 mL with 70% ethanol (EEP) in a volumetric flask. From each crude sample, three parallel extracts with 70% ethanol were prepared according to the method of Popova et al.[12]. The solutions were evaporated to near dryness on a rotary evaporator under reduced pressure at 40℃and then freeze-dried. The percentages of balsam in the extracts were calculated as the ethanol soluble fraction.

The specific absorbance of UV spectrum of EEP was obtained according to the method of Miyatakaet al.[13]. The UV absorption spectra of EEP and their maximum absorption (λmax) were measured with a MAPADA UV-1600PC ultraviolet-visible spectrophotometer (Shanghai, China).

TP content of EEP was determined by using the Folin-Ciocalteu method [15], adapted to a micro scale. In a 15mL centrifuge tube, 8.9mL distilled water, 0.1mL sample appropriately diluted and 0.4mL Folin-Ciocalteu reagent was added and vortexed. After exactly 1min, 0.6mL of sodium carbonate (20%)was added and then the mixture was vortexed. The absorbance was read at 750nm after 120min reaction at room temperature in obscurity. Results were expressed as mg/mL gallic acid equivalent (GAE).TF content was determined as follows: EEP (0.3mL)was diluted with 70% aqueous ethanol (2.7mL). An aliquot of 1.0mLwas added to test-tubes containing 0.2mL of 10% aluminum nitrate, 0.2mL of 1M aqueous potassium acetate and 8.6mL of 70% ethanol. The absorbance was read at 415nm after 40min reaction at room temperature[4].Results were expressed as mg/mL quercetin equivalent (QE).1Mililiter of EEP and 2mL of DNP solution (1g DNP in 2 mL 96% sulfuric acid, diluted to 100mL with methanol in a volumetric flask)were heated at 50℃ for 50min in a water bath. After cooling to room temperature, the mixture was diluted to 10mL with 10% KOH in methanol (w/v) and 0.1mL of the resulting solution was added to 4.9mL methanol. Absorbance was measured at 486 nm[12],[16].Calibration (r2=0.9967)was performed by using naringen in as reference. Results were expressed as mg/mL naringenin equivalent (NE).

In order to establish the EC50, the percentage of DPPHRSA was determined for every propolis sample at different concentrations[12],[17].The reaction mixtures in the tube consisted of sample (0.05mL) and DPPHR (2.9mL, 0.1mM) dissolved in ethanol. The absorbance was measured at 517nm against a blank. The percentage of SA was calculated as $[1-(A1 - A2)/A0] \times 100\%$, where A0is the absorbance of the control, A1is the absorbance of the sample and A2is the absorbance of blank that contained sample without DPPHR. The SA of the samples is expressed as the EC50 value which was the concentration required to scavenge 50% of DPPHR.

The liquid chromatographic system used for determination of propolis in EEP was an Agilent 1100 Technology. Chromatographic separations were performed with a YMC-Pack ODS-A analytical column, 4.6×150mm L.D., 5μm particle size, purchased from YantaiZhenghai Electronic Mask Co., Ltd, Yantai, China. The temperature of the HPLC column was kept at 20℃. Acetonitrile was used as the mobile phase of isocratic elution at a flow rate of 0.6mL/min. The injection volume was set to 10 μL. The individual compounds in propolis were determined quantitatively according to the absorbance at 292nm.

The mobile phases consisted of 0.1% aqueous formic acid (solvent A) and acetonitrile (solvent B).

Gradient elution was as follows: start at 20% B; 0−15min increase via linear gradient to 30% B; 15−20min increase via linear gradient to 35% B; hold for 15min;35−55min increase via linear gradient to 55% B; hold for 10min; 65−80min, increase via linear gradient to 100% B. All 8 compounds were determined quantitatively against external standards. Quantification was based on peak area. Calibration curves of the standards were made by diluting stock standards in methanol.

The antioxidant activity of propolis samples at different concentrations (0.02%, 0.05%, 0.10%) in lard was determined by Rancimat (Metrohm, Switzerland) based on the method published by Huangetal. [18]. The air flow rate was controlled at 20 l/h, the temperature was controlled at 100°C and lard was used as the substrate. Lard (3 ±0.02 g) and different levels of antioxidants were added to each sample. Each sample was prepared in duplicate. The protection factors (PF) were calculated according to the following formula: PF=(IP sample)/(IP control). The lard without the addition of antioxidant served as the control. The lard applied in this method was rendered in the laboratory from fresh pig fat tissue.

Data were reported as the mean ± SD for triplicate determinations. Unmodified method, which was principal components analysis (PCA), is applied to observe patterns in the data indicating relationships between samples and/or between variables. Statistics were analyzed using SPSS for Windows (version rel. 10.0.5, 1999, SPSS Inc., Chicago, IL, USA). Statistical significance is declared at $P<0.05$.

3. Results and discussion

As shown in Table 1, the TP content was found to vary from 16.76 to 243.61mg GAE/g propolis. The TP content of fifteen propolis samples from China varied from 87.11 to 257.93 mg GAE/g propolis. The highest contents were close, but the lowest contents of TP in our research were much lower than that of previous report. This may be due to the number of propolis samples in our research, which are much more than those reported by Shi et al.[13]. It is also important to know that the propolis samples collected in our research are scattered all over mainland of China as Figure S1 shows. As shown in Table 1, the TF content varies from 13.43 to 732.80 mg QE/g propolis and the TDF content varies from 17.36-185.80mg NE/g propolis. Higher TP, TF and TDF contents are observed in EEP from Central and East China.

Table 2 shows that TP content correlate highly ($p<0.01$) with the contents of TF ($r = 0.892$) and TDF($r = 0.815$). TP content correlate highly ($p<0.01$) with the contents of 3,4-dimethoxycinnamic acid ($r = 0.820$), chrysin ($r = 0.755$) and naringenin ($r = 0.729$) while TF content correlate highly ($p < 0.01$) with naringenin ($r = 0.774$). Consequently, TDF also correlated highly ($p<0.01$) with naringenin ($r = 0.788$) and cinnamic acid ($r = 0.745$). Here, the correlation is better when the r value is larger.

As shown in Table 1, the $E_{1cm}^{1\%}$ values of most samples are 180-400 and the λ_{max} of most samples are 290-293 nm. These values were near to those previously reported [6],[14],[19]. The $E_{1cm}^{1\%}$ values correlated highly ($p < 0.01$) with the content of TP ($r = 0.810$), TF ($r = 0.740$) and TDF ($r = 0.710$) in this research (Table 2). Furthermore, $E_{1cm}^{1\%}$ values have significant statistically correlation with TP, TF, TDF contents in our research, so it could be used as an indicator to estimate the quality of propolis.

As shown in Table 1, the balsam content of raw propolis samples ranges between 8.13 and 79.42%.The distribution of black squares in Figure1G shows that the balsam content correlates ($p < 0.01$) with latitude of geographical region where the propolis was collected ($r = - 0.633$)and its mathematical relationship is expressed as follows: $Y = -1.5538X + 100.3550$ (X is latitude; Y is balsam content). Butthe samples from Kunming Yunnan, Gaoan Jiangxi, Anshun Guizhou, Yueyang Hunan, Tunchang Hainan, Jianweng Fujian, Hangzhou Zhejiang, Hengyang Hunan, Hengyang Hunan, which are shown by blank triangles, don't conform to the formula. The terrain and environment of the nine samples is complex. Most of them were collected in the Southern part of China, which mostly have high altitude. For example, Kunming Yunnan and Anshun Guizhouare located in the Yunnan-Guizhou Plateau while Jianweng Fujian and Hangzhou Zhejiang are located in the Wuyi Mountains. The samples from Hengyang Hunan, Hangzhou Zhejiang and Tunchang Hainan were collected on the mountains. Interestingly, the balsam content of propolis from the highlands of China, represented by blank triangles as said earlier correlated highly($p < 0.01$) with latitude ($r = -0.645$), and the mathematical relationship is as follows: $Y = -1.8905X + 83.2382$ (X is latitude; Y is balsam), this also shows that the balsam content of propolis decreases as the latitude increases (Figure1G).

Samples from high latitude (northern region) and high altitude(mountains and plateaus in the southern region of China) had significantly lower concentrations of bioactive components in poplar-type propolis. This was similar to what was reported by Kalogeropoulos*et al*.[1] and Popova *et al*.[12]. The bioactive components decrease as balsam content decreases, and this is demonstrated by a significant positive correlation, i.e. the balsam content correlated highly ($p < 0.01$) with the content of TP ($r = 0.755$), TF ($r = 0.773$), TDF ($r = 0.803$)

in this research (Table 2). High percentage of balsam content meant that the propolis contains a low percentage of wax and insoluble substances. Propolis with high balsam content has a higher content of bioactive components (i.e. TP, TF and TDF contents) and this result agrees highly with one reported by Kujumgiev*et al.*[4] and Popova *et al.*[12].

It means that balsam content was not only influenced by latitude but also by high elevation because phytocoenosiums changed greatly according to the latitude of the lands. The content of balsam extracted from crude propolis is an important characteristic in determining the correlation between balsam content and bioactive components.

The propolis sample C14 had the strongest DPPHR SA, with EC_{50} values of 1.11mg the raw propolis/mL. The bioactive components of propolis initially showed a negative correlation with the EC_{50} values of DPPHRSA, which was not straightforward for the discussion of standard. Therefore, the reciprocal of EC_{50} values of DPPHRSA (i.e. $1/EC_{50}$) was used instead (Table 2). Table 2 shows that $1/EC_{50}$expresses superior positive correlation with TP ($r = 0.818$, $P<0.01$) but inferior positive correlation with TF ($r = 0.757$, $P <0.01$) and TDF ($r = 0.692$, $P <0.01$). The high DPPHRSA of propolis was most likely attributed to TP content because its coefficient of correlation was the largest. Previous studies also showed that there was a strong positive correlation between antioxidant activities and TP content, and TF content largely influenced the antioxidant activities of propolis collected in Brazil[20] It had a similar conclusion for propolis in China in our present research. Furthermore, our research results show that there is also a strong positive correlation between DPPHRSA and the contents of TDF in propolis. DPPHRSA of propoliscould be used as an important indicator to estimate the quality of EEP. The individual components also correlated highly ($p<0.01$) with DPPHRSA. DPPHRSA even correlated better with 3,4-dimethoxycinnamic acid ($r = 0.767$) and chrysin ($r = 0.660$) than others (Figure S2).

To compare the chemical composition of the Chinese propolis samples, the eight compounds contents were determined by HPLC in 85 min, based on propolis weight (Table 3). The seeight compoundsare caffeic acid (A), p-coumaric acid (B), ferulic acid (C), isoferulic acid (D), 3,4-dimethoxycinnamic acid (E), cinnamic acid (G), naringenin (I) and chrysin (K), and their chemical structures are shown in FigureS3. Figure2ashows typical HPLC chromatograms of propolis. The HPLC chromatograms of propolis sample C15, C26, C7, C4 and C14 collected from China are similar，but different from those of samples C54 and C55 collected from Brazil and Madagascar respectively. The contents of eight compounds in these seven propolis samples were expressed in the histogram as shown in Figure2b and these compound contents from China present certain regularity. As shown in Figure 1A-F, chrysin content correlates highly ($p < 0.01$) with caffeic acid($r=0.789$),p-coumaric acid ($r=0.692$), ferulic acid ($r=0.765$), isoferulic acid ($r=0.804$) and 3,4-dimethoxycinnamic acid contents ($r=0.855$). Cinnamic acid content correlates highly ($p < 0.01$) with naringen in content ($r=0.944$). The relationship between the individual phenols in propolis has never been reported yet in authors' knowledge scopes.

Relationship between the contents of chrysin, p-coumaric acid, ferulic acid and isoferulic acidin Brazilianpropolis sample C54was not in conformity with those in Chinese propolis. The contents of eight compounds in Madagas carpropolis C55were too low to be compared with other samples. As a result of the relationship between the contents of chrysin and p-coumaric acid, ferulic acid andisoferulic acidfound in the Chinese propolisand Brazilian propolis, the Madagascar propolis could be easily distinguished. Relationship between cinnamic acidand naringenin contents in Brazilian propolis is in conformity with those in Chinese propolis. Relationship between cinnamic acid and naringenin contents provides the basis for the quality control of propolis

The large number of analyzed samples gives us the unique opportunity to statistically characterize poplar-type propolis with respect to its bioactive (antioxidant and antibacterial) components: TP, TF and TDF. Although the chemical composition of poplar bud exudates was relatively constant, there could be significant variations in the percentage of individual constituents in distinct locations or even in materials from different individual plants [21]. These variations reflect in propolis composition, shown in the histograms (Figure3). The large difference of propolis in China as shown clearly in Figure 3makesthe establishment of the standard of Chinese propolis difficult.

As observed in Figure 3,statistical analysis of the distribution was normal and 20% Empirical quantiles as the Table 2 shown is used to set the minimum values for the contents of balsam,bioactive components and DPPHR SA. Choosing the 10% quantile would lead to lowvalues for balsam, bioactive components and DPPHRSA. According to the results, the following characteristics for a typical poplar propolis sample can be used as a basis for standardization and quality control: balsam, minimum 30%; total phenols, minimum 108 mg GAE/g; TF, minimum 209 QE/g; TDF, minimum 69 NE/g; $E^{1\%}_{1cm}$ value, minimum 260and DPPHRSA($1/EC_{50}$), minimum 206 $(g/mL)^{-1}$.

The six minimum values, i.e. $E_{1cm}^{1\%}$,$1/EC_{50}$, balsam content, TP content, TF content and TDF content in propolis were given. Due to these high variations in values of propolis from different geographic origins and their consequent complications in practical applications, the PCA was used as unsupervised pattern recognition to reveal the possible relationships between the studied variables. One PC(Principal Component)was constructed to relate the contents of balsam, TP, TF,TDF, the values of $E_{1cm}^{1\%}$ and DPPHRSA, which represents 74.29% of the variability. The equation is as follows：

PC (Principal Component) = $0.957*\lambda_1 +0.935*\lambda_2 + 0.899*\lambda_3 + 0.881*\lambda_4+0.603*\lambda_5+0.881*\lambda_6$,(where,$\lambda_1$=TP content, λ_2=TF content, λ_3=TDF content,λ_4=DPPH radical scavenging activity ($1/EC_{50}$), λ_5= $E_{1cm}^{1\%}$ value and λ_6=balsam content).20% Empirical quantiles is used to set the minimum values for PC, namely 833(Table2).

The bioactive components and DPPHRSA of Madagascar propolis sample C55werenot only lower than the mean values of propolis from China, but were also lower than the minimum values (Table1 and Table 2). The contents of TF and TDF, $E_{1cm}^{1\%}$ value and DPPHRSA of sample C55wereobviouslylower than the lowest values of Chinese propolis (Table1). The HPLC chromatogram pattern of sample C55was very different from those of Chinese propolis. Isoferulic acid and 3,4-dimethoxycinnamic acid were not detected in sample C55 from Madagascar and the contents of other compounds were very low as shown by HPLC analysis. On the contrary, the chemical compositions and DPPHRSA of propolis from Brazil were not only higher than the minimum values of Chinese propolisbut were even higher than the mean values of the propolis from China except for TDF content (Table1 and Table2).

Our results show that measuring the contents of groups of active compounds of propolis, rather than individual components, is the appropriate approach to develop quality standards for propolis. It agrees with the report by Popova *et al*.[12]. Our results also indicate that measurement of DPPHR SA should be an obligatory element in quality control of propolis because of the contents of TP, TF and TDF are important bioactive components which are good for health. The results obtained in our research show that the chosen parameters are meaningful for the evaluation of poplar-type propolis quality. It is important to remember however, that other types of propolis have different chemical composition[3]. For this reason, all the above conclusions and criteria are valid for poplar propolis only, and should by no means be applied to other propolis types, such as Brazilian green propolis or Cuban and Brazilian red propolis[12]. Furthermore, the conclusions and criteria were valid for the propolis in China, because the samples were all collected in China. But it also has value to be referenced by the samples from Asia, Europe or the regions in temperate zone.

One unexpected finding is that *Apissinensis* cannot produce propolis, but *Apismellifer*a L. can.It is extremely interesting that we cannot collect any propolis from *Apissinensis* (means China bees or Chinese species, originated from China) although there are a lot of *Apissinensis*found all over China.All propolis samples are collected from *Apismellifera* (Italianbees). So, it is not surprising that we cannot find any description about the medicinal usage of propolis in a very famous antique Chinese traditional medicine book, "*Compendium of Materia Medica*" [22], which is regarded as the Bible of Chinese Traditional Medicine. In this book, all the products of bee, including honey, royal jelly, honeycomb, baby bee,bee venom and bee wax can be used as Chinese Traditional Medicine and their medicinal properties are described in details[23]. It is true that China did notintroduce European bee in Li Shizhen's time. We have carefully done the survey whether *Apissinensis produce propolis or not* together with professor Zhang Huaiqiong and Professor Jiang Jian who are Traditional Chinese Medicine experts from Shanghai University of Traditional Chinese Medicine and further recognize that *Apissinensis* cannot produce propolis.

The effect of antioxidants on the oxidative of lard was evaluated by PF. As shown in Table 4, when the concentrations were 0.02%, the PF of most propolis samples were smaller than that of BHT (PF=2.33). When the concentrations were 0.05%, the PF of part propolis samples were smaller than that of BHT (PF=3.40). When the concentrations were 0.10%, the PF of 4propolis samples were smaller than that of BHT(PF=3.60). Among the 4 propolis samples, C40、C52 was collected from China andC54、C55 was collected from Brazil andMadagascar, respectively. It means that the propolis samples have remarkable antioxidant activity in oil, as well as antioxidant effect increased sharply with increasing concentration.

This study clearly demonstrates that the contents of the groups of bioactive components correlated highly with their DPPHRSA and among these, the TP content positively correlatedbest with DPPHRSA.The individual components contents correlated highly with DPPHRSA and3,4-dimethoxy- cinnamic acid content positively have the strongest correlation with DPPHRSA Balsam content correlated highly with TP, TF and TDF contents in our research. The balsam content correlated highly with latitude and the balsam content of propolis decreases as the latitude increases. It means that the balsam content is not only influenced by latitude but also by high

elevation. According to our result, the set minimum value for PC was 833, which can be used as a basis for standardization and quality control. Relationship between cinnamic acid and naringenin contents also provides the basis for the quality control of propolis. The propolis samples have remarkable antioxidant activity in oil, as well as antioxidant effect increased sharply with increasing concentration.

Acknowledgments

The authors thank Prof. Youhua He (Department of mathematics, Faculty of Sciences, Shanghai University) for the constructive suggestion in selection of mathematical models.We also thank Prof. Zhang Huaiqiong and Prof. Jiang Jian (Shanghai University of Traditional Chinese Medicine) for final recognization of that *Apissinensis* cannot produce propolis.

Table 1 Contents of Balsam, TP, TF, TDF, Specific Absorbance ($E_{1cm}^{1\%}$), DPPHR SA(EC_{50})of Propolis Collected in Different Regions[a].

Code	Collectionarea	Balsam (%)	TP (mgGAE/g) [b]	TF (mgQE/g)	TDF (mgNE/g)	$E_{1cm}^{1\%}$ (λ_{max})	EC_{50} (mg/mL)
C1	Xuchang, Henan	44.87±0.45	189.87±0.38	532.24±1.48	108.91±0.85	405.31 (292)	3.36±0.001
C2	Kunming, Yunnan	29.76±0.88	114.48±0.23	189.13±1.31	70.69±0.85	354.86 (291)	**3.07±0.004**
C3	Fuyang, Anhui	59.12±0.43	193.88±0.39	557.10±1.19	155.13±0.53	345.93 (292)	2.06±0.002
C4	Jiangyin, Jiangsu	62.82±0.6	228.64±0.97	570.36±1.42	136.02±0.26	406.42 (292)	1.66±0.003
C5	Beipei, Chongqing	48.35±1.34	112.61±0.74	121.17±0.87	58.24±0.37	368.50 (289)	3.69±0.0009
C6	Baoji, Shanxi	71.05±0.85	239.60±0.88	702.97±0.98	156.47±0.49	372.30 (292)	1.92±0.003
C7	Linfen, Shanxi	53.73±0.23	200.83±0.12	567.05±0.31	119.58±0.61	377.14 (292)	2.39±0.002
C8	Qingzhou,Shandong	52.32±0.68	213.40±0.43	499.09±0.40	119.13±1.00	355.43 (292)	2.04±0.003
C9	Liaocheng,Shandong	54.00±0.51	209.92±1.68	560.42±0.29	132.24±1.67	**346.76 (292)**	2.38±0.007
C10	Gaoan, Jiangxi	38.48±1.38	165.28±1.32	354.88±0.27	86.91±0.16	377.05 (293)	2.92±0.007
C11	Xinmi, Henan	29.15±1.17	107.80±0.86	278.63±0.70	67.58±0.67	306.21 (292)	3.47±0.002
C12	Anshun,Guizhou	40.92±0.63	136.14±1.09	240.51±0.38	112.24±0.36	312.46 (290)	3.39±0.001
C13	Yueyang, Hunan	27.35±0.72	136.14±0.59	306.81±0.35	69.80±0.34	340.89 (292)	3.98±0.008
C14	Anqing, Anhui	**79.42±0.67**	234.79±0.46	620.09±0.57	150.69±1.06	373.22 (292)	**1.11±0.000**
C15	Rizhao, Shandong	61.88±0.60	222.76±1.78	550.47±0.20	163.80±0.70	373.04 (292)	2.24±0.001
C16	Jincheng, Shanxi	44.50±0.30	198.70±1.59	499.09±0.60	113.80±0.54	355.39 (292)	2.47±0.45
C17	Chengdu, Sichuan	43.83±1.68	216.34±0.43	593.57±0.61	107.58±0.90	490.49 (291)	3.02±0.002
C18	Xiangyang, Hubei	37.67±0.71	157.52±0.32	**366.48±0.75**	110.24±0.96	349.38 (292)	3.95±0.001
C19	Anyang, Henan	40.08±0.38	106.73±0.21	414.55±0.01	80.69±0.87	145.78 (269)	5.84±0.007
C20	Tunchang, Hainan	46.32±0.86	173.56±0.35	291.89±0.20	94.69±0.54	417.75 (292)	2.48±0.25
C21	Huanggang, Hubei	57.10±0.73	134.53±0.27	424.50±0.78	110.47±0.35	324.34 (292)	1.82±0.002
C22	Siping, Jilin	36.49±0.35	133.46±0.27	295.21±0.28	71.36±0.78	265.42 (291)	3.41±0.005
C23	Qingzhou, Shandong	43.32±0.83	209.66±0.42	522.29±1.68	144.24±0.68	505.93 (292)	2.53±0.006
C24	Yantai, Shandong	45.72±0.16	162.87±0.33	404.61±1.35	92.02±0.29	329.62 (292)	2.99±0.001
C25	Qinzhou, Guangxi	61.03±0.89	49.25±0.10	33.32±1.19	71.36±1.25	**9.58 (287)**	15.93±0.003
C26	Huizhou, Guang dong	53.92±1.03	221.15±0.44	596.88±1.34	137.13±0.42	401.71 (292)	2.34±0.005
C27	Raohe, Heilongjiang	32.69±0.95	111.54±0.22	182.50±0.72	72.02±0.58	314.47 (293)	4.23±0.007

C28	Yuling, Shanxi	48.03±2.07	178.91±0.72	338.31±0.41	79.36±0.50	289.51 (292)	2.18±0.005
C29	Xinyang, Henan	48.73±0.30	194.42±0.78	639.15±0.72	166.69±0.44	410.40 (292)	2.86±0.002
C30	Yining, Xinjiang	40.77±0.32	146.83±1.09	414.55±0.01	106.69±1.65	350.04 (290)	2.64±0.001
C31	Jianweng, Fujian	40.98±0.08	115.28±0.30	378.09±0.41	105.80±1.12	451.89 (292)	5.32±0.003
C32	Liaoyang, liaoning	27.58±0.29	**138.27±0.55**	213.99±0.67	66.47±0.56	385.92 (292)	3.36±0.002
C33	Hangzhou, Zhejiang	20.95±0.53	62.61±0.25	106.25±0.53	52.02±1.21	189.74 (290)	10.85±0.007
C34	Wutaishan, Shanxi	30.77±0.50	131.59±0.53	160.95±1.00	73.80±1.31	342.90 (292)	3.52±0.001
C35	Linfen, Shanxi	28.93±0.20	111.81±0.22	320.07±1.91	98.24±0.91	332.31 (292)	6.59±0.002
C36	Yushan, Jiangxi	47.35±0.13	153.65±0.31	389.69±1.23	121.36±0.86	377.51 (292)	4.74±0.003
C37	Longnan, Gansu	35.33±0.50	111±0.22	248.80±1.12	108.02±0.14	366.51 (291)	3.62±0.009
C38	Baotou, Neimeng	25.23±0.48	77.32±0.15	187.47±0.63	64.69±0.25	258.39 (291)	10.62±0.890
C39	Jingning, Gansu	27.48±0.28	94.70±0.19	272.00±0.30	66.91±0.32	216.86 (292)	6.25±0.060
C40	Jinan, Shandong	35.18±1.00	63.68±0.13	180.01±0.17	83.80±0.10	134.3 (292)	12.38±0.003
C41	Dezhou, Shandong	39.05±0.09	148.43±1.88	423.67±0.45	116.02±0.22	335.08 (292)	3.24±0.006
C42	Yili, Xinjiang	31.01±0.95	132.39±0.26	303.50±0.87	**99.58±0.16**	315.68 (292)	2.18±0.002
C43	Hengyang, Hunan	26.34±0.28	100.84±0.71	283.61±1.56	88.69±0.19	323.3 (292)	3.61±0.009
C44	Taoyuan, Hunan	67.53±0.32	**243.61±1.71**	732.80±1.20	183.80±0.22	398.32 (292)	1.69±0.001
C45	Zhuzhou, Hebei	39.49±0.72	158.59±1.11	400.46±0.08	124.69±0.14	373.50 (292)	3.87±0.005
C46	Dulan, Qinghai	37.88±0.23	131.06±0.92	372.29±1.78	110.91±0.54	305.61 (292)	2.91±0.001
C47	Meixian, Guangdong	76.95±0.28	188.27±1.32	**699.65±0.03**	**185.80±0.32**	227.68 (292)	1.65±0.001
C48	Hengyang, Hunan	24.56±1.11	31.60±0.22	**13.43±0.84**	**17.36±0.33**	14.25 (292)	**27.26±0.003**
C49	Fengxian, Shanghai	**40.39±0.80**	138.54±0.46	484.17±0.66	74.47±0.29	377.72 (292)	3.02±0.007
C50	Jixian, Tianjing	28.49±0.12	106.06±0.23	276.15±0.56	39.80±0.46	**595.70 (292)**	4.28±0.001
C51	Changbaishan, Jilin	47.52±0.83	126.38±0.48	485.83±0.72	68.91±0.36	196.21 (292)	2.35±0.007
C52	Yulin, Guangxi	**8.13±0.18**	**16.76±0.40**	15.91±0.23	27.58±0.69	43.08 (292)	15.82±0.002
C53	Wangzhai, Shanxi	32.02±0.55	131.59±0.92	278.63±0.17	50.24±0.36	307.34 (292)	3.02±0.002
C54	Belo Horizonte, Brazil	63.39±1.04	171.02±1.20	453.51±0.26	64.02±0.63	354.33 (292)	2.65±0.003
C55	Madagascar, Africa	17.53±0.38	24.65±0.17	23.37±0.13	20.47±1.33	33.08 (292)	72.17±0.002

[a]Data are reported on a per gram of propolis basis as the mean ± SD (n = 3). [b]GAE-gallic acid equivalent; QE-quercetin equivalent; NE-naringenin equivalent. The minimal, maximal, and median values of each column are shown in bold type. EC_{50} values are the effective concentration (mg/mL) at which 50% of DPPHRis scavenged.

Table 2 Characteristics (DPPHRSA ($1/EC_{50}$), the Contents of Balsam, TP, TF and TDF)of PropolisSamples Collected from Different Regions of China and the Correlation (r^a)between these Characteristics

Characteristics	Data analysis								Correlation (r^a)					
	Mean Value	Max Value	Min Value	P90	P80	P20	P10		$1/EC_{50}$[b] $(g/mL)^{-1}$ $(g/mL)^{-1}$	TP[b] (mg GAE/g)	TF[c] (mg QE/g)	TDF[c] (mg NE/g)	Balsam[c] (%)	$E^{1\%}_{1cm}$[c]
$1/EC_{50}$ $(g/mL)^{-1}$	329.22	900.90	5.14	538.00	448.89	206.37	92.96		1.000	0.818 **	0.757 **	0.693 **	0.780 **	0.742 **
TP (mg GAE/g)	147.46	243.61	16.76	222.11	202.60	107.58	69.14			1.000	0.892 **	0.815 **	0.755 **	0.810 **
TF (mg QE/g)	375.38	732.80	13.43	610.81	557.76	209.02	137.08				1.000	0.853 **	0.773 **	0.740 **
TDF (mg NE/g)	99.90	185.80	17.36	155.93	133.00	68.64	54.51					1.000	0.803**	0.710 **
Balsam (%)	42.25	79.42	8.13	62.44	53.94	29.95	26.80						1.000	0.622 **
$E^{1\%}_{1cm}$	320.59	595.70	9.58	413.34	384.28	259.80	141.18							1.000
PC	1072.13	2026.04	93.11	1645.04	1488.46	833.21	518.98		- -					

[a]Correlation is better when r is larger.

[c]Values significantly different by bivariate correlations test: **, P<0.01

Table 3 The PF[a] values of propolis samples in different concentrations （M±SD，n=3）[b]

Code	Pf		
	0.02%	0.05%	0.10%
BHT	2.33±0.01	3.4±0.89	3.60±0.27
C1	2.88±0.72	5.25±0.28	8.62±0.63
C2	2.44 ±0.62	4.67 ±0.05	7.61 ±0.36
C3	2.28±0.42	4.61±0.74	8.52±0.45
C4	2.27±0.56	3.79±0.38	6.25±0.27
C5	2.50±0.24	4.47±0.49	7.56±0.18
C6	2.16±0.02	3.61±0.14	5.90±0.45
C7	2.79±0.67	5.51±0.05	8.05±0.14
C8	3.11±0.08	5.77±0.34	7.77±0.18
C9	2.73±0.34	5.32±0.08	8.86±0.32
C10	2.92±0.05	5.28±0.27	8.51±0.24
C11	1.94±0.18	3.37±0.49	5.03±0.24
C12	1.84±0.38	2.97±0.38	4.78±0.08
C13	1.77±0.08	4.21±0.05	7.42±0.62
C14	2.38±0.27	3.97±0.62	6.59±0.79
C15	2.37±0.44	4.35±0.46	7.21±0.65
C16	2.23±0.16	2.43±0.78	7.96±0.32
C17	1.59±0.94	2.44±0.08	4.12±0.27
C18	2.99±0.38	5.38±0.27	9.4±0.05

C19	2.67±0.38	2.37±0.05	4.23±0.38
C20	2.72±0.05	3.81±0.36	6.33±0.52
C21	1.69±0.66	2.56±0.08	3.83±0.14
C22	2.05±0.05	3.37±0.49	7.02±0.23
C23	3.33±0.71	6.63±0.22	10.38±0.18
C24	2.52±0.24	5.74±0.16	10.10±0.87
C25	2.37±0.45	4.4±0.24	7.32±0.38
C26	1.96±0.23	4.03±0.18	6.56±0.71
C27	1.20±0.05	1.45±0.18	4.90±0.23
C28	2.54±0.12	3.61±0.05	6.25±0.05
C29	1.78±0.18	3.23±0.71	5.18±0.16
C30	1.54±0.87	2.46±0.45	4.07±0.27
C31	1.53±0.36	2.49±0.71	3.64±0.24
C32	2.30±0.38	3.91±0.27	6.53±0.38
C33	1.67±0.23	2.56±0.16	4.63±0.18
C34	1.95±0.62	3.43±0.38	5.56±0.14
C35	2.27±0.18	4.00±0.24	5.87±0.05
C36	2.18±0.05	5.33 ±0.15	8.12 ±0.05
C37	1.74±0.87	3.36±0.36	5.78±0.23
C38	1.71±0.18	3.01±0.45	4.26±0.23
C39	1.91±0.22	3.38±0.38	6.99±0.05
C40	2.30±0.49	2.14±0.62	2.01±0.27
C41	1.36±0.5	3.43±0.1	5.91±0.28
C42	2.6±0.14	4.48±0.02	7.27±0.45
C43	1.95±0.22	3.43 ±0.25	5.56 ±0.05
C44	1.69±0.14	3.35±0.23	5.19±0.15
C45	2.39±0.24	4.44±0.14	9.66±0.21
C46	1.68±0.08	2.89±0.16	4.97±0.03
C47	1.60±0.02	2.81±0.11	7.64±0.23
C48	1.21±0.08	1.98±0.04	3.89±0.71
C49	1.79±0.36	2.94±0.06	7.33±0.14
C50	2.61±0.24	4.58±0.18	8.66±0.24
C51	2.88±0.71	4.53±0.24	8.66±0.27
C52	1.10±0.02	1.64±0.16	2.01±0.18
C53	2.74±0.18	4.98±0.14	9.35±0.36
C54	1.26±0.02	1.22±0.14	2.01±0.02
C55	0.87±0.05	1.11±0.08	1.47±0.08

[a] PFis short for the protection factors
[b] Values are reported as the mean ± SD on raw propolis weight basis (n = 3).

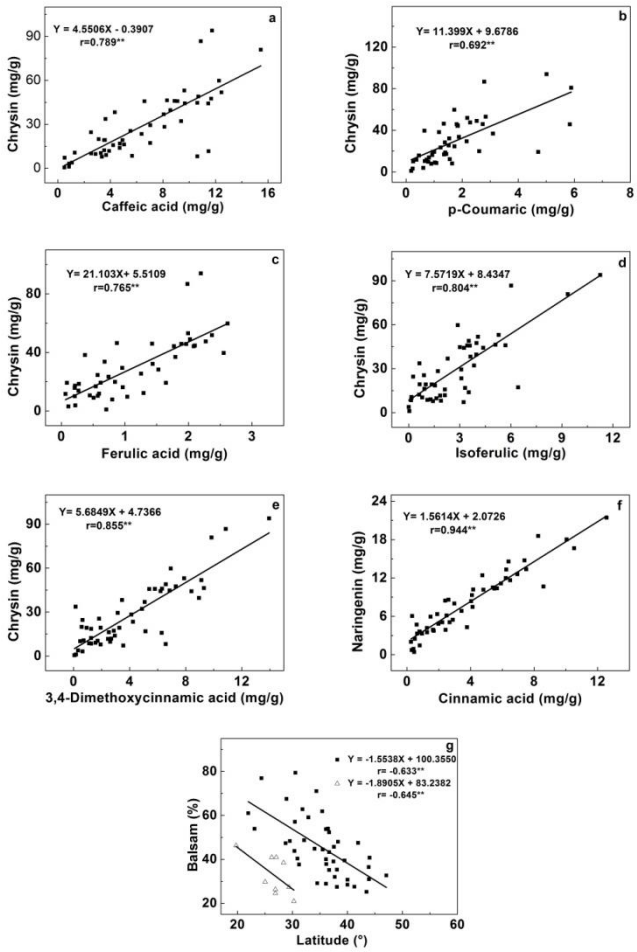

Figure 1. Relationshipsbetween compounds. A. relationshipbetween caffeic acid and chrysin; B. relationshipbetween p-coumaric and chrysin; C. relationshipbetween ferulic acid and chrysin; D. relationshipbetween isoferulic acid and chrysin; E. relationshipbetween3,4-dimethoxycinnamic acid and chrysin; F. relationshipbetweencinnamic acid and naringenin;G. relationship between latitude and balsam; The relationship between latitude and balsam is linear relation. The blacksquares represent for the group one and its mathematical relationship is expressed as follows: Y = -1.5538X + 100.3550 (X is latitude; Y is balsam content). The blank triangles represent for the group two and its mathematical relationship is expressed as follows the mathematical relationship was as follow: Y = -1.8905 X + 83.2382 (X is latitude; Y is balsam);∗∗P < 0.01

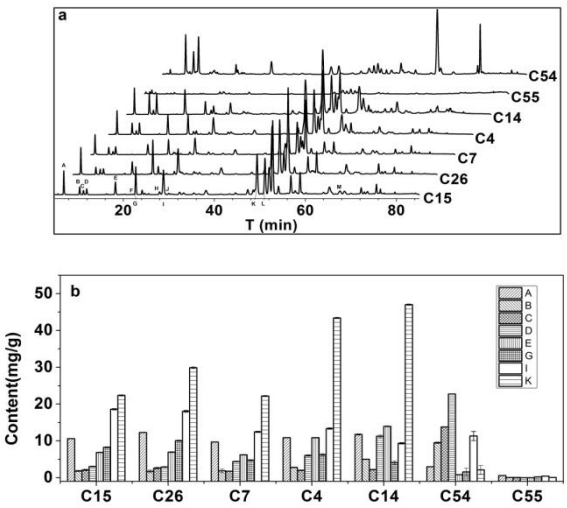

Figure2.a. Representative HPLC chromatograms of different propolis samples collected from different regions. b.The content of 8 compounds in propoliscollected from different regions.A. caffeic acid; B. p-coumaric acid; C. ferulic acid; D. isoferulic acid; E. 3,4-dimethoxycinnamic acid; F. quercetin; G. cinnamic acid; H. apigenin; I. naringenin; J. kaempferol; K. chrysin; L. pinocembrin; M. pinostrobin. Compounds A-E, G, I and K had been determined quantitatively. Compounds F, H, J, L and M had been determined qualitatively.

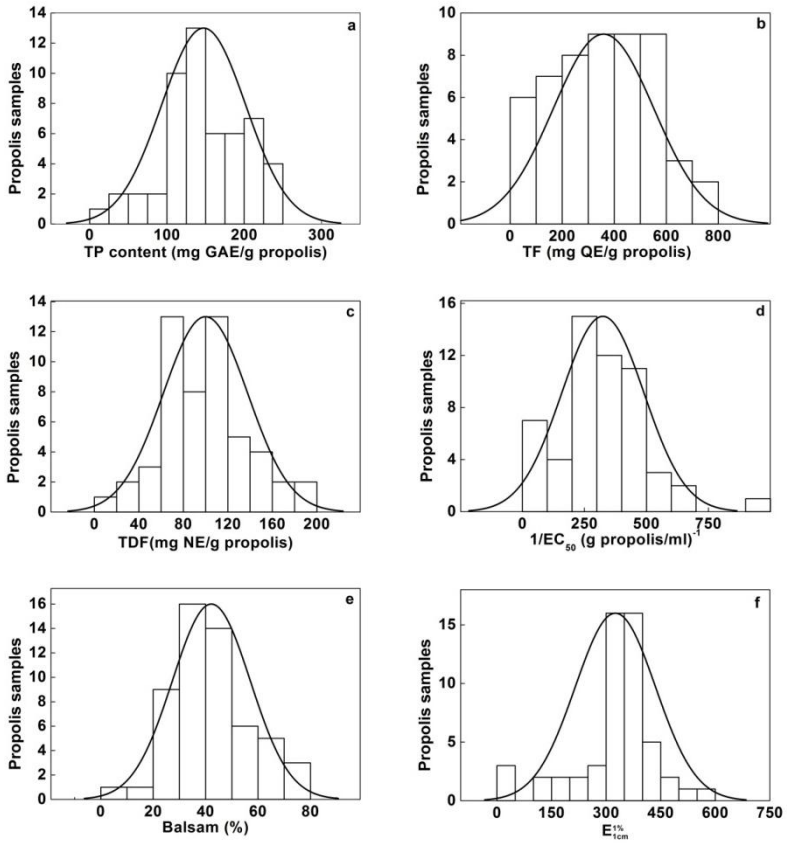

Figure3. Histograms of the characteristics of poplar propolis (53 samples): A. Distribution of TP contents; B. Distribution of TF content; C. Distribution of TDF content; D. Distribution of $1/EC_{50}$; E. Distribution of total balsam content; F. Distribution of $E_{1cm}^{1\%}$.

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