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Polyphenol Content in Chinese Bee Products and Their Antioxidant Activity

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Considering gallic acid as the standard, a spectroscopic method is established for the determination of the polyphenol contents in bee products (6 kinds of propolis capsules, 3 kinds propolis tablets and 12 kinds bee pollen). The scavenging capacity of samples to DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals was studied. The results show that the average content of polyphenol in capsule is 30.49 %, in tablet is 27.01 %, in bee pollen is 3.67 %; the removing ability to DPPH free radicals of propolis capsule and its tablet are stronger than bee pollens, there is obvious linear relationship between the total polyphenol content and its ability of scavenging free radicals in certain concentration.

Key Words: Bees products, Propolis, Pollen, Total polyphenols, Antioxidant activity.

INTRODUCTION

Propolis is a gelatinous substance formed the process that bees repeated inject their gland secretions into the resin material which they collect from the new branches buds, bud and trauma of the glue source plant. Bee pollen is a granular aggregates after bees injecting some nectar and saliva into the collected pollen. Both propolis and bee pollen are the natural active substances of fauna and flora that have unique double medicinal efficacy. They are rich in flavonoids, polyphenols, amino acids, vitamins, enzymes, minerals and other chemical ingredients. Known as the "natural micro-nutrient library", they have the functions of antioxidant, improving cardiovascular circulation, strengthening the immune system, protecting the prostate and digestive system[1-4]. In recent years, with the development of bee industry and the increase of people's awareness of health care, the propolis products (mainly propolis capsules and propolis tablets) which are made up of propolis raw material and bee pollen health products are favoured by the markets. Although there are some quality standards about propolis and its extraction in China, they determine the total flavonoid content of the sample by taking rutin as the quality control indicator and there is no uniform standard of quality control on propolis products and bee pollen[5-9].

Considering that the propolis is one of the natural products which contains the most total phenol[10], in this paper we take gallic acid as the standard, utilize tartaric acid ferrous spectrophotometry to determine the polyphenol content in bee products and takes spectrometry to study the DPPH radical scavenging capacity of samples to character the antioxidant capacity of various bee products. We expects to provide more scientific basis for in-depth development and utilization of series of health bee products.

EXPERIMENTAL

The propolis capsule, propolis tablet and bee pollen for the experiment are bought from bee product companies in Anhui, Gansu, Chongqing, Guangdong, Yunnan, Shanghai, Xinjiang, Beijing and Brazil. The products' raw materials are from Anhui, Gansu, Chongqing, Guangdong, Yunnan, Qinghai, Xinjiang, Jiangxi, Shandong and Hebei. They are numbered according to the product forms.

UV-Visible spectrophotometer (USA Aglient 8453); electronic analytical balance (Ohaus International Trade Co., Ltd.);ultrasonic cleaner of KQ-600DB type (Kunshan Ultrasonic Instrument Co., Ltd.); gallic acid reference substance (content ≧ 99.0 %) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH, content ≧ 99.0 %) are purchased from Sigma Company, sodium tartrate, ferrous sulphate, disodium hydrogen phosphate, potassium dihydrogen phosphate and anhydrous ethanol are analytically grade; all aqueous solution were prepared with water (Wahaha , Hangzhou,China).

Preparation of analytical reagent: Preparation of ferrous tartrate solution: take 0.5001 g ferrous sulphate and 2.5002 g potassium sodium tartrate, put them in a 500 mL volumetric

flask, dissolve them with purified water and constant volume to the graduation, shake the solution and make cryopreservation for spare.

Preparation of the phosphate buffer: Mixed 0.0667 mol/L disodium hydrogen phosphate solution with 0.0667 mol/L potassium dihydrogen phosphate solution at a proportion of 84:16 and regulate the pH to 7.5 for reserve.

Preparation of gallic acid standard solution: Weighed 25 mg gallic acid standard and put it in 100 mL volumetric flask, dissolved it with purified water to the graduation and shake up. The 0.25 mg/mL gallic acid standard solution was produced and make cryopreservation for spare.

Preparation of DPPH standard solution: Weigh 8.2 mg DPPH accurately, dissolve it with anhydrous ethanol and set the constant volume in 250 mL volumetric flask, store in the dark for reservation (use it right after it is ready).

Preparation of samples: Respectively take 5 g propolis capsules powder and propolis tablets, prepare their polyphenol extracts according to the reference[11]. Dissolve proper amount of these extracts with anhydrous ethanol in 10 mL volumetric flask and shake up for reservation. Weigh 50 mg bee pollen accurately and use ultrasonic extraction for 0.5 h in anhydrous ethanol, filter in 10 mL volumetric flask for later use.

Determination of polyphenol content of samples: Take appropriate test solutions respectively and place them in 25 mL volumetric flask; add distilled water to 5 mL and followed by the addition 5 mL ferrous tartrate solution. Set the constant volume to 25 mL with phosphate buffer solution of pH 7.5. Shake up and stand for 15 min. Measure the absorbance of the test samples and the blank solution at 540 nm with the same method.

Standard curve of gallic acid: Accurately measured 1, 2, 3, 4 and 5 mL of gallic acid standard solution and put them in 25 mL of volumetric flasks, respectively; add distilled water to the volume of 5 mL and add 5 mL ferrous tartrate solution; make the constant volume to 25 mL with phosphate buffer solution of pH 7.5. Shake up and stand for 15 min. Measured the sample solutions absorbance at 540 nm and measure the absorbance of the blank solution without gallic acid with the same method. Take the concentration of gallic acid solution as the abscissa and absorbance value and draw standard curve of the linear regression equation: A = 0.5747X – 0.0246, the correlation coefficient r = 0.9995.

DPPH standard curve: Transfer 0, 1, 3, 4, 5, 6, 7, 8, 9 and 10 mL quasi reserve liquid into 10 mL volumetric flasks, dilute to the constant volume with absolute alcohol to get the series of standard solution. Determine the absorbance of the standard solution at 517 nm wavelength and draw standard curve.

Measurement of DPPH solution stability performance and reaction time: The scavenging action of the samples to DPPH can be dynamically monitored by measuring the changes of the solution optical density at 517 nm wavelength with ultraviolet-visible spectrometer. Take the extract of number one capsule as the research objective of the correlation between DPPH removal rate and reaction time. Add 1 mL sample solution to 9 mL DPPH ethanol solution and mixed thoroughly. Determine D values of this solution after every 30 s until D value reaches steady state.

DPPH free radical scavenging ratio of samples: Determine the absorbance of the sample solution when their DPPH radical scavenging reaction reaches equilibrium, the DPPH free radical scavenging ratio of propolis products and bee pollen in different concentrations can be calculated by the following formula:

$$\text{Scavenging ratio} = \left(\frac{A_0 - A}{A_0}\right) \times 100\ \%$$

where A_0 = absorbance of DPPH solution = absorbance of DPPH solution with antioxidants.

Calculation of the IC_{50} value in the DPPH free radical scavenging of samples: The DPPH free radical scavenging capacity of antioxidants can be expressed in terms of IC_{50} (the free radical scavenging concentration under the free radical scavenging rate of 50 %). The smaller the value is, the more significant the scavenging effect will be. Make the sample into solution of serial concentrations; work out the regression equation of every sample and calculate the IC_{50} value of each sample solution through these equations.

RESULTS AND DISCUSSION

Content of polyphenols in propolis products and bee pollen: The drug health care efficacy of bee products is closely related to the compositions and the content of polyphenols (including flavonoids and phenolic acids), which are the material basis of efficacy and health care effect of bee products, so the total polyphenols content can be used as one of the quality control indexes of bee products[10].

As is illustrated in Tables 1 and 2, the polyphenols content are very differences when the propolis products origin from different producing area, different factories and different plant variety. There is no significant difference in the polyphenols content of the same kind of plants in different regions and different kinds of plants in the same region, for example, the polyphenol content in the bee pollen of rape flowers ranges from 3.5-3.8 %, which is in close proximity to the average value (3.67 %) and far less than that in propolis products (27-30.5 %). The results show that in the propolis products and bee pollen the polyphenols show obvious difference in content.

Stability of the DPPH solution and the confirmation of reaction time: The reaction kinetics research of propolis capsule clearing DPPH free radical shows that, in the first 5 min when the antioxidant is added to DPPH solution, its optical

TABLE-1
TOTAL POLYPHENOL CONTENT AND DPPH FREE RADICAL REMOVAL ABILITY OF PROPOLIS PRODUCTS

Sample numbers	Polyphenol content (g/100 g)	IC_{50} (μg/mL)
Propolis capsule 1	23.46	22.59
2	32.39	64.71
3	24.83	89.80
4	40.55	33.77
5	29.11	95.93
6	32.61	26.09
Average value	30.49	–
Propolis tablets 7	25.94	78.15
8	37.35	32.27
9	17.75	15.77
Average value	27.01	–

TABLE-2
TOTAL POLYPHENOL CONTENT AND DPPH FREE RADICAL REMOVAL ABILITY OF BEE POLLEN

Sample numbers	Polyphenol content (g/100 g)	IC_{50} (μg/mL)
Rape bee pollen 1	3.52	0.29
2	3.50	0.38
3	3.64	0.28
4	3.56	0.28
5	3.70	0.25
6	3.25	0.35
7	3.80	0.28
(Average value 3.57)		
Rose bee pollen 8	3.81	0.74
9	5.52	31.87
Camellia bee pollen 10	3.24	1.30
Herba leonuri bee pollen 11	2.97	11.98
Schizandra bee pollen 12	3.57	0.32
Grand average	3.67	–

density (D) drops more rapidly, as time goes on, the downtrend of optical density gradually tends to be flat and reach steady state 40 min later, as is shown in Fig. 1. Therefore, we use the D value at the time of 40 min after the reaction of the sample with DPPH solution as the observation point.

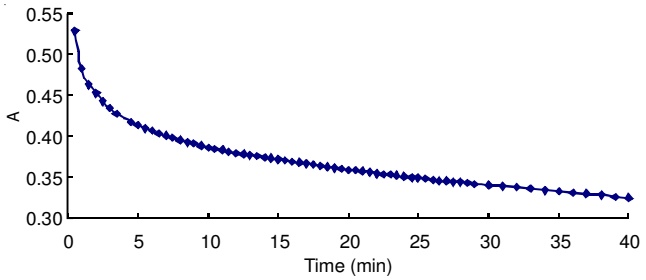

Fig. 1. DPPH light density changing with time

Scavenging capacity to DPPH free radicals of bee products: The scavenging capacity to DPPH free radicals of samples can be represented as IC_{50}, the smaller the value of IC_{50} is, the lower the concentration of oxidants needed will be. As shown in Tables 1 and 2, in Figs. 2 and 3, the scavenging capacity of different bee products is related to the total content of polyphenols and give a liner a relationship in certain concentration; the average polyphenol content of propolis products is higher than the bee pollen *ca.* 10 times, their ability to remove free radicals is *ca.* 10 times then late. However, there is a great difference in the antioxidant capacity of bee pollen from different kinds of plants, the bee pollen of rape flowers owns the best performance. Propolis products are rich sources of flavonoids phenolic, unsaturated fatty acids, carotene, vitamin A, vitamin E and vitamin C. The synergistic effect of these substances contributes to the antioxidant capacity of propolis products. The chemical composition and antioxidant capacity of propolis products varies due to the difference in their sources. The experimental results suggest that not only the content of polyphenols and flavonoids phenolic but also the contribution of other substances to bioactivities should be taken into account.

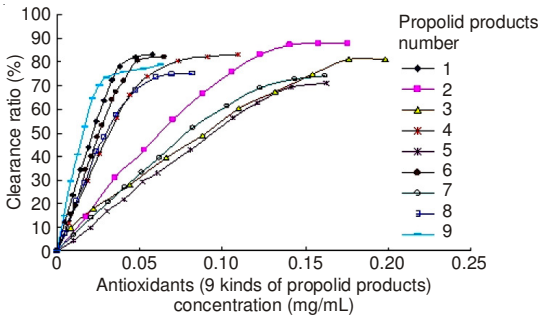

Fig. 2. Antioxidant capacity of 9 kinds of propolid product in different concentrations

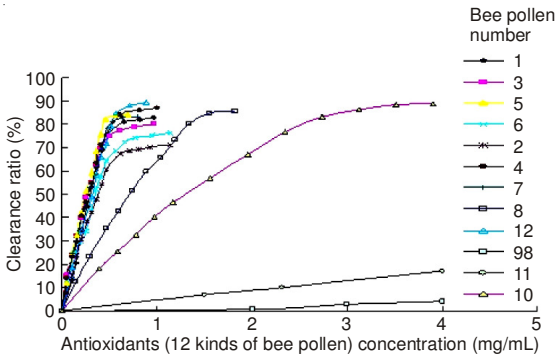

Fig. 3. Antioxidant capacity off 12 kinds of bee pollen in different concentrations

Conclusion

Determination of polyphenol content in bee products-propolis capsules,propolis tablets and bee pollen have shown that the average content of polyphenols in propolis products is *ca.* 30 %, which is far more than that in bee pollen (*ca.* 4 %). With the scavenging capacity to DPPH free radicals eepresents the antioxidant capacity of samples and the results illustrate that propolis products own greater capacity to clear free radicals than bee pollen. There are some dramatic correlation between the total polyphenol content and the antioxidant capacity in bee products. The antioxidant capacity of bee products are the results of synergistic effect of different kinds of substances, so it is necessary to go into the material basis of the antioxidant capacity and the inner link and obtain in-depth knowledge of the antioxidant mechanism in them.

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