

## A survey of the incidence of poplar tree gum in propolis products on the Chinese retail market

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## ORIGINAL RESEARCH ARTICLE

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Propolis has multiple biological activities, and the high demand for it and limited availability have led to adulteration by poplar tree gum. In this survey, we used salicin as a marker to monitor the incidence of poplar tree gum in propolis semi-products or final products circulated in the Chinese market, using an HPLC method. A total of 67 samples were tested, and poplar tree gum was not detected in 23 of these samples. However, 66% of the samples did contain poplar tree gum. Of 50 finished propolis products from 26 manufacturers, salicin was present in 27 products from 13 manufacturers. The survey showed that products from the same manufacturer contained either real propolis or poplar tree gum product. Moreover, the concentrations of salicin varied in commercial propolis products from different manufacturers. This proposed method can be effectively used for detecting poplar tree gum in propolis products.

E

El propóleo presenta una actividad biológica múltiple. Debido a la alta demanda y a la limitada disponibilidad, los productos de propóleos se suele adulterar con goma de álamo. En este ensayo, se usó salicina como marcador para monitorizar la incidencia de la goma de álamo en los productos finales e intermedios de propóleos que circulan por el mercado chino, mediante el uso de cromatografía líquida de alta eficacia (HPLC). De un total de 67 muestras que fueron examinadas, en 23 no se detectó goma de álamo. Sin embargo, el 66% de las muestras sí contenían goma de álamo. De 50 productos de propóleos procesados de 26 fabricantes, la salicina estaba presente en 27 productos de 13 fabricantes. De este modo, el ensayo demuestra que productos de un mismo fabricante pueden contener o bien propóleo o bien goma de álamo. Por otra parte, las concentraciones de salicina variaron en los productos de propóleo de los diferentes fabricantes. Este método puede ser usado eficazmente para detectar goma de álamo en productos de propóleo.

K : propolis; poplar tree gum; salicin; adulteration

I

Propolis is a resinous substance collected by bees from various tree buds, such as poplar, birch, beech, horse chestnut, alder, and various conifers (Bankova et al., 1992; Ghisalberti, 1979; Marcucci, 1995). Bees mix the original resin with their salivary and enzymatic secretions (Bankova, de Castro, & Marcucci, 2000). The resulting material is one of the few natural remedies that have been employed extensively since ancient times. Currently, herbalists recommend propolis for its anti-bacterial, anti-fungal, anti-viral (Ghisalberti, 1979; Marcucci, 1995), hepatoprotective (Bhadauria, 2012; González et al., 1995; Lin, Lin, Chen, Chung, & Sau, 1997), anti-inflammatory (Borrelli et al., 2002; Hu et al., 2005; Park, Kim, & Park, 1996; Paulino et al., 2003; Tan-No et al., 2006), and immunostimulating (Fischer et al., 2007; Manolova, Maksimova, Manolova, Stoilova, & Korchak, 1987) properties to increase the body's natural resistance to infections. Therefore, the use of propolis as a health food supplement has increased to promote health and prevent diseases all over the world (Burdock, 1998).

However, the collection and harvesting processes for propolis are slow and costly. The high demand and limited availability of authentic propolis motivate some manufacturers to adulterate propolis products with a much cheaper material, namely poplar tree gum. Poplar tree gum, which is the artificially brewed extract of *Populus* buds, leaves, bark and other tissues, has a colour, smell, chemical compositions and antimicrobial activity similar to those of the poplar-type propolis (Vardar-Ünlü, Silici, & Ünlü, 2008), and it has been extensively used all over the world since the late 1990s because of its low cost and ready availability. The practice of obtaining cheaper poplar tree gum allows some companies to be more profitable than those companies selling pure propolis products. In addition, customs authorities have reported that the disturbing trend of the export of poplar tree gum from China to countries and territories abroad has rapidly increased, indicating that China has become a base for global propolis counterfeiting. Although the adulteration may be not unhealthy, consumers' confidence is usually influenced by these frauds, resulting in fewer sales of pure products.

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Over the past decade, methods to distinguish propolis from poplar tree gum have attracted great attention. Anti-counterfeiting analytical methods range from simple colorimetric methods to more sophisticated techniques, such as liquid chromatography (Zhou, Chen, Hu, Hu, & Shao, 2009), Fourier transform infrared spectra, and two-dimensional infrared correlation analysis (Wu, Sun, Zhao, Li, & Zhou, 2008). Nevertheless, there is still a lack of sensitive and selective analytical methods to distinguish propolis from poplar tree gum.

Our previous research presented an “authenticity factor”, namely salicin, to distinguish poplar tree gum from propolis. Salicin is found in poplar tree gum, but not in propolis, because it is hydrolysed by  $\alpha$ -glucosidase from honey bees during propolis collection and processing (Zhang, Zheng, & Hu, 2011a; Zhang, Zheng, Liu, & Hu, 2011b). A simple and reliable HPLC method has been developed for detecting the presence of salicin in poplar buds, leaves and poplar tree gum. For this method, the sample is simply extracted with 75% ethanol. The analytical method has been proven to be an effective approach for the rapid analysis of poplar tree gum due to its high sensitivity and selectivity (Zhang et al., 2011b).

Propolis is composed of 50% resin, 30% wax, 10% essential oils, 5% pollen and 5% various organic compounds. It cannot be used as crude material, but must be purified by extraction with the proper solvents. The ethanol extract or extract powder is the most common commercially available as a semi-finished product. It is profitable for manufacturers to purchase semi-finished products and use them to manufacture finished products. Finished products (either in pure form or combined with *Astragalus membranaceus* or pollen) are currently available in the market and are sold as capsules, liquids (hydroalcoholic) and tablets. The present study surveyed the authenticity of the semi-finished and finished propolis products circulating in the Chinese market, using HPLC, with salicin as the reference standard.

## M

### Chemicals

The salicin standard (M.W. 286;  $\geq 98\%$  chemical purity) was purchased from Sigma-Aldrich (China) and used for the preparation of calibration curves and in sample recovery experiments. High purity solvents (HPLC grade acetonitrile and methanol) were obtained from Merck (Germany). HPLC grade water was purified by a Yjd-upws ultra-pure water system (China). Absolute alcohol and phosphoric acid were analytical grade. A stock standard solution of salicin was prepared by adding an accurately weighed amount of salicin (10 mg) to a 10 ml flask and diluting it with the appropriate volume of methanol, and the stock solution was stored at  $-4^\circ\text{C}$ . Quantitation of salicin was achieved using a corresponding calibration curve. A fresh diluted working standard solution was prepared weekly for calibration.

The genuine propolis product samples (ethanol extracts, powders, soft capsules, hard capsules, pills and liquids) and poplar tree gum samples used during method development were provided by the China Bee Products Association (Beijing, China). Sixty-seven commercial products, including six Brazilian green propolis products from four different companies, were purchased from retail outlets, and these commercial products were manufactured by twenty-six of the most qualified brand manufacturers in China. All samples were stored in dark and dry places at room temperature ( $20\text{--}30^\circ\text{C}$ ).

### Sample preparation

The propolis products were presented in the form of ethanol extracts, powders, soft capsules, hard capsules, pills or liquids. Liquid samples (1 ml) were simply diluted to 5 ml with 75% aqueous alcohol. The ethanol extracts (0.5 g), granular powders (0.5 g), crushed pills (20 pills), opened hard capsules (10 capsules) and opened soft capsules (10 capsules) were dissolved in 25 ml of 75% aqueous alcohol and then treated as liquid samples. After dilution, the resulting solutions were extracted in an ultrasonic water bath for 30 min at room temperature. The extracted solutions were then centrifuged at 4,000 g for 15 min at room temperature, and the supernatant was collected. The extract was filtered through a 0.45  $\mu\text{m}$  membrane filter unit. Finally, 5 l of each sample solution was analysed by HPLC.

### HPLC analysis

HPLC determination was performed with an Agilent 1200 series instrument equipped with a vacuum degasser (G1322A), a quaternary pump (G1311A), an autosampler (G1329A), a programmable variable wavelength detector (VWD; G1314B), and a thermostatted column compartment (G1316A). A Sepax HP-C18 analytical column (150 mm  $\times$  4.6 mm; 5  $\mu\text{m}$ ) was used at  $30^\circ\text{C}$  with a flow rate of 1 ml/min. The gradient consisted of isocratic conditions at A (0.5% aqueous phosphoric acid) and 5% B (acetonitrile) for 13 min, a linear gradient to 80% B over 1 min. After isocratic conditions at 80% B for 10 min a linear gradient back to 5% B over 1 min was applied. Detection was performed with an ultraviolet detector set at 213 nm.

### Method validation

To assess the overall validity, analytical parameters, such as linearity, the limits of detection (LOD) and quantification (LOQ), and precision, repeatability and recovery of salicin for different products were determined. Recovery using the proposed method was performed with blank samples and the standard addition method. The ethanol extract, powder, liquid, pills, soft capsules and

hard capsules of real propolis were used. Moreover, each authentic propolis product was spiked with three different concentration levels (0.02, 0.04 and 0.08 mg/ml) of salicin with six parallels at each level. The samples were then prepared as described above. The precision of the method was estimated by evaluating the intra-batch precision and inter-batch precision. The intra-batch precision was examined by five replicate analyses of the same sample solution on the same day, and the inter-batch precision was determined twice a day over three independent days. The relative standard deviation (RSD) was calculated as a measure of precision. Because ethanol extracts are the most common semi-finished materials and are used in different finished products, the detection limit of poplar tree gum in the propolis ethanol extracts was also determined.

## R

### Method validation

The linearity of the analytical method was determined by means of calibration curves. A regression line was fitted by applying the linear regression model based on the least square method. Based on a six point calibration, a linear response was observed in concentration range from 10 to 100 g/ml for salicin, with correlation coefficients over 0.9997.

LOD was measured as the lowest amount of the salicin that may be detected to produce a response that is different from that of a blank ( $S/N = 3$ ). The salicin concentration obtained was 1.3 g/ml. LOQ was measured as the lowest amount of salicin that can be reproducibly quantified above the baseline noise ( $S/N = 10$ ). The salicin concentration obtained was 5.4 g/ml.

The mean recovery ranges of salicin in the low, intermediate and high spiked levels were 98.8–104.5, 97.8–107.2, 98.1–106.4, 99.2–105.6, 98.4–101.7 and

97.3–102.7% for the propolis ethanol extract, powder, soft capsules, hard capsules, liquid and tablets, respectively (Table 1). Table 2 summarizes the intra-batch precision and inter-batch precision for the ethanol extract, powder, liquid, pills, soft capsules and hard capsules of propolis preparations containing poplar tree gum. The RSD values of the intra-batch and inter-batch precision for all type of products were less than 1.23 and 1.57%, respectively.

Figure 1 shows the HPLC chromatograms obtained for the propolis ethanol extracts with 0, 5, 10, 20, 30 and 40% poplar tree gum. Compared to the genuine product samples, salicin was easily detected when the ethanol extract of propolis was adulterated by more than 10% poplar tree gum. Therefore, the detection limit of poplar tree gum in commercial propolis ethanol extracts was estimated to be as low as 10%.

### Sample analysis

Data for the incidences and levels of salicin (the marker compound in the poplar tree gum) in the 67 samples are shown in Table 2. Salicin was undetected or non-quantifiable in 34% of the analysed samples (23 of 67 samples). Salicin was detected in 64% of the ethanol extract samples. Salicin was detected in four powder and four pill samples representing 67% of the retail propolis powder or tablet samples. Adulteration was found in 17 of the soft capsule samples (63%) with measurable levels of 0.011–0.029 mg/grain (30%) and obviously measurable levels greater than 0.04 mg/grain (33%). The hard capsule samples had the lowest incidence of adulteration, with salicin obviously measurable in only four samples, representing 57% of the retail propolis hard capsules. The liquid samples had the highest incidence of adulteration with salicin obviously measurable in eight samples representing 80% of the retail propolis liquid.

Table 1. Recovery of salicin in various propolis preparations.

Sample category	Theoretical concentration (mg/ml)	Concentration found $\pm$ SD (mg/ml) <sup>a</sup>	RSD (%)	Recovery (%)
Ethanol extract	0.02	0.0198 $\pm$ 0.0003	1.45	98.8
	0.04	0.0410 $\pm$ 0.0007	1.63	102.6
	0.08	0.0836 $\pm$ 0.0014	1.72	104.5
Powder	0.02	0.0214 $\pm$ 0.0003	1.43	107.2
	0.04	0.0391 $\pm$ 0.0004	1.12	97.8
	0.08	0.0818 $\pm$ 0.0015	1.89	102.3
Soft capsule	0.02	0.0204 $\pm$ 0.0003	1.45	102.3
	0.04	0.0392 $\pm$ 0.0005	1.32	98.1
	0.08	0.0851 $\pm$ 0.0015	1.81	106.4
Hard capsule	0.02	0.0198 $\pm$ 0.0003	1.44	99.2
	0.04	0.0417 $\pm$ 0.0005	1.27	104.3
	0.08	0.0845 $\pm$ 0.0010	1.16	105.6
Liquid	0.02	0.0203 $\pm$ 0.0003	1.69	101.7
	0.04	0.0393 $\pm$ 0.0005	1.38	98.4
	0.08	0.0798 $\pm$ 0.0010	1.28	99.8
Tablet	0.02	0.0195 $\pm$ 0.0003	1.53	97.3
	0.04	0.0411 $\pm$ 0.0007	1.70	102.7
	0.08	0.0789 $\pm$ 0.0012	1.52	98.6

<sup>a</sup>Values represent the means  $\pm$  SD of six samples per concentration.

Table 2. Inter- and intra-batch precision of salicin in various propolis preparations adulterated by poplar tree gum.

Sample category	Inter-batch precision (n = 5)		Intra-batch precision (n = 6)	
	Measured concentration (mean $\pm$ SD) (mg/ml)	RSD (%)	Measured concentration (mean $\pm$ SD) (mg/ml)	RSD (%)
Ethanol extract	0.0146 $\pm$ 0.0001	0.74	0.0148 $\pm$ 0.0002	1.03
Powder	0.0253 $\pm$ 0.0003	1.03	0.0255 $\pm$ 0.0003	1.37
Soft capsule	0.0239 $\pm$ 0.0003	1.15	0.0231 $\pm$ 0.0004	1.57
Hard capsule	0.0230 $\pm$ 0.0003	1.23	0.0229 $\pm$ 0.0003	1.30
Liquid	0.0257 $\pm$ 0.0002	0.81	0.0253 $\pm$ 0.0003	1.06
Tablet	0.0380 $\pm$ 0.0004	0.94	0.0374 $\pm$ 0.0004	1.05

Among the different types of analysed propolis products with poplar tree gum, the salicin concentrations presented a large degree of variability. The salicin concentrations varied by 6-, 2-, 12-, 2-, 3- and 5-fold in the propolis ethanol extracts, powders, soft capsules, hard capsules, liquid samples and tablets, respectively.

#### **Distribution of adulterated samples among participating companies**

Salicin was obviously measurable in 27 samples from 13 different manufacturers purchased at local grocery stores indicating the presence of poplar tree gum in

these samples. Salicin was measurable in six samples from four different manufacturers and undetected or non-quantifiable in 17 samples from nine different manufacturers indicating low concentrations or the absence of poplar tree gum in these products. Different types of products from certain manufacturers were either real propolis or contained poplar tree gum product.

#### **D**

Regarding the recognition of adulterated propolis products, detection of poplar tree gum can be easily

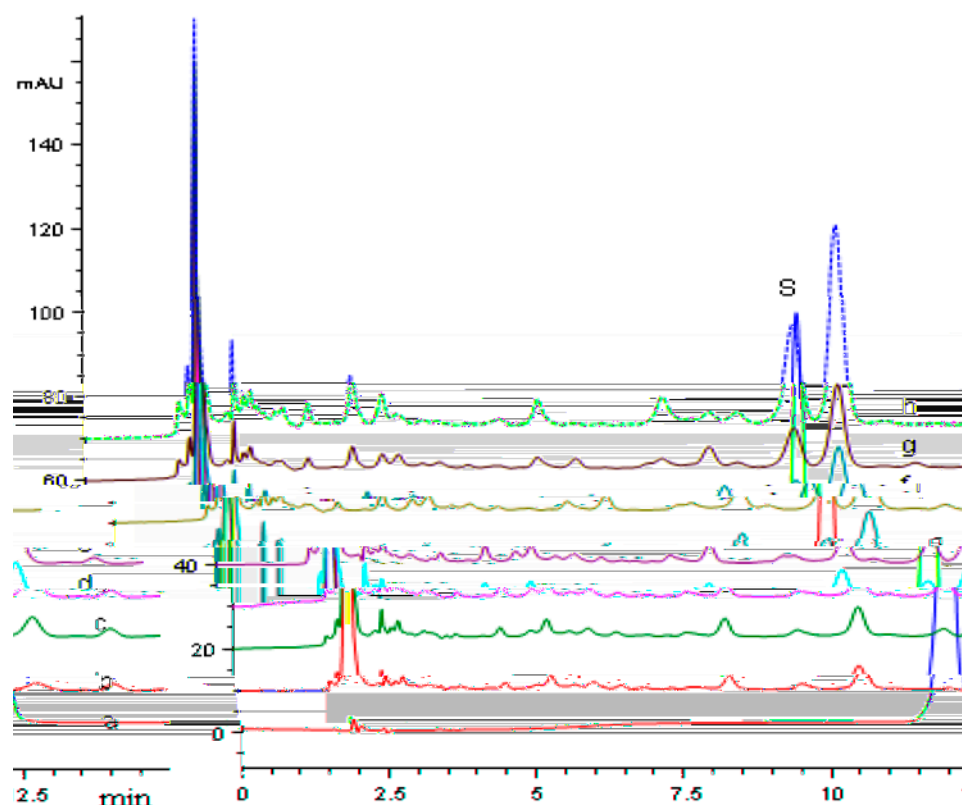


Figure 1. HPLC profiles of a pure poplar tree gum ethanol extract and propolis ethanol extracts with 0, 5, 10, 20, 30, and 40% poplar tree gum.

Notes: The labels indicate the following samples: (a) salicin; (b) ethanol extract of real propolis; (c) ethanol extract of propolis adulterated by 5% poplar tree gum; (d) ethanol extract of propolis adulterated by 10% poplar tree gum; (e) ethanol extract of propolis adulterated by 20% poplar tree gum; (f) ethanol extract of propolis adulterated by 30% poplar tree gum; (g) ethanol extract of propolis adulterated by 40% poplar tree gum; and (h) poplar tree gum.

Table 3. Salicin concentration in various propolis products as measured by HPLC.

Sample category	Analysed samples	Positive sample	Salicin in positive samples		
			Mean $\pm$ SD	RSD (%)	Range
Ethanol extract	11	7	0.93 $\pm$ 0.61 mg/g	65.4	0.34–2.0 mg/g
Powder	6	4	0.39 $\pm$ 0.09 mg/g	23.4	0.29–0.50 mg/g
Soft capsule	27	17	0.05 $\pm$ 0.04 mg/capsule	79.1	0.011–0.13 mg/capsule
Hard capsule	7	4	0.05 $\pm$ 0.02 mg/capsule	33.4	0.033–0.063 mg/capsule
Liquid	10	8	0.16 $\pm$ 0.07 mg/ml	43.7	0.095–0.29 mg/ml
Tablet	6	4	0.04 $\pm$ 0.02 mg/tablet	61.1	0.014–0.072 mg/tablet

performed under the proposed experimental conditions. Salicin was found in the analysed adulterated propolis products, but the relative concentrations of salicin in different product types and products produced by various manufacturers were quite variable. These differences may be caused by differences in the amount of raw materials in different product types and different sources of poplar tree gum.

The profile generated from this survey can be integrated into a validated method for detecting adulteration in propolis products. In this survey, salicin was undetected in only 17 of the 50 analysed commercial product samples. Moreover, salicin was detected in trace amounts in approximately 12% of the analysed samples. We hypothesized that these products were most likely not adulterated by the manufacturers but that the raw materials purchased from the suppliers were contaminated by poplar tree gum because most of these manufacturers lack effective detection and monitoring systems to distinguish between poplar tree gum and real propolis.

Recently, the public media in China has reported some cases of poplar tree gum adulteration in propolis, indicating that this situation is common and serious. The present survey supported these concerns because approximately 54% ( $n = 50$ ) of the samples collected from 26 of the most qualified brand manufacturers in China were detectably adulterated with poplar tree gum. These concerns are further validated when considering that more than 1,000 small manufactures produce or sell propolis products in China. The proposed method in this survey is practical and reliable. Of the commercial products tested in this study, only 34% of the products qualified as authentic propolis products (Table 3). Therefore, it is necessary to develop criteria for the quality of propolis to regularly monitor the incidence of poplar tree gum in raw materials, semi-products and retail products to guarantee the rights and interests of consumers and manufacturers of propolis products.

In the present study, we proposed a simple, convenient and effective method to detect the commercial sources of products containing added poplar tree gum by comparing retentions, UV-vis spectra and HPLC profiles of salicin in semi-product materials and finished products. The contents of salicin can be accurately

determined in poplar tree gum. The present results showed that poplar tree gum posing propolis is a common and serious problem. This is the first report of adulterated propolis products in which added poplar tree gum was matched to its commercial source. The current survey will provide useful information for formulating authenticity criteria in propolis products.

## D

No potential conflict of interest was reported by the authors.

## F

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## R

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