Food Chemistry 119 (2010) 907–912

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The content of astilbin and taxifolin in concentrated extracts of *Rhizoma Smilacis Glabrae* and turtle jelly vary significantly

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ARTICLE INFO

Article history:
Received 19 March 2009
Received in revised form 8 June 2009
Accepted 28 July 2009

Keywords:
Astilbin
Taxifolin
Turtle jelly
Capillary electrophoresis
Food quality

ABSTRACT

Extract of *Rhizoma Smilacis Glabrae* (*RSG*) is one of the main ingredients in turtle jelly, which is a traditional functional food in Southern China and Hong Kong. A capillary electrophoresis method was successfully developed for determination of astilbin and taxifolin in turtle jelly and *RSG* concentrated extract samples. For six determinations of astilbin and taxifolin at concentrations of 20 μg ml^{-1}, the relative standard deviations of migration time were 0.62% and 0.87%, while those of the peak area ratios were 2.17% and 3.62%, respectively. Eighteen turtle jelly samples manufactured in mainland China and Hong Kong were collected for analysis. The results show that astilbin and taxifolin were all from the *RSG* ingredient. The contents of astilbin and taxifolin in turtle jelly were distinctly different between brands, and some were found not to contain these substances.

1. Introduction

Turtle jelly, also called Gui-ling-gao in Chinese, is a traditional functional food popular in Southern China and Hong Kong. Turtle shell and *Rhizoma Smilacis Glabrae* (*RSG*) are the two main ingredients in turtle jelly. The food is made by stewing the turtle shell for about 20 h, and then the stew is added to a broth made from many herbs including *RSG*. The result is a bowl of steaming, quivering black goo. This food is said to be hepatoprotective, and can minimise the effects of damp-heat, nourish yin and clear toxins in the blood. It is specifically claimed to improve skin disorders. However, to the best of our knowledge, turtle jelly is not mentioned in the scientific literature. Thus, the claimed health benefits of this food may be just folklore or an advertising ploy of manufacturers and so lack a scientific base.

Although turtle shell may provide many nutrients such as protein and minerals, it is believed that the therapeutic effects of turtle jelly, if any (e.g. skin disorders improvement), are most likely due to the herbal additives. As the main ingredient of turtle jelly, we have previously analysed the constituents of *RSG* by capillary electrophoresis. The results showed that astilbin is the main constituent in the herb with contents ranging from 1–4%, while taxifolin is also present (Zhang, Li, Lai, & Cheung, 2009). Astilbin is a dihydroflavonol rhamnoside with antioxidative (Zhang, Zhang, & Cheung, 2009), antibacterial (Moulari et al., 2006) and hepatoprotective activities (Closa et al., 1997; Wang, Zhao, & Xu, 2004; Xu et al., 1999). Previous studies showed that astilbin could inhibit lymphocyte migration and suppress delayed-type hypersensitivity, negatively regulate the activity of cytokine and inhibit contact hypersensitivity (Cai, Chen, & Xu, 2003; Fei, Wu, & Xu, 2005). It may also have significance in the treatment of immunologically related diseases (Yan & Xu, 2001). Taxifolin is the aglycone of astilbin. Studies show that it can reduce cerebral ischemic reperfusion injury in rats through its antioxidative effect (Wang et al., 2006). It can also up regulate phase II detoxification enzymes through an antioxidant response element in HCT 116 cells (Lee, Cha, Selenge, Solongo, & Nho, 2007). Using HepG2 cells as a model, Theriault et al. (2000) demonstrated that taxifolin could decrease hepatic lipid synthesis with a concomitant decrease in apolipoprotein B. Further research showed that taxifolin reduced apolipoprotein B secretion by limiting triglyceride availability (Casaschi, Rubio, Maiyoh, & Theriault, 2004).

As turtle jelly is a functional food containing the decoction of large amounts of *RSG*, it is very important to determine the content of astilbin and taxifolin to prove its functional profile. In this work, a simple and rapid capillary zone electrophoresis method for simultaneous determination of astilbin and taxifolin was developed. Different brands of turtle jelly sample collected from mainland China and Hong Kong were analysed. Furthermore, three concentrated extracts of *RSG* produced by different pharmaceutical companies were also analysed and compared. Our results reveal that the quality of these functional foods,

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doi:10.1016/j.foodchem.2009.07.048

in terms of the contents of astilbin and taxifolin, varied significantly.

2. Experimental

2.1. Chemicals and materials

Astilbin (>99%) was a gift from Chen (Nanjing University, China). Taxifolin (>85%), borax and β-cyclodextrin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Throughout the study Milli-Q deionised water was used. It was prepared by a Milli-Q water system (Millipore, MA, USA). Different brands of turtle jelly sample were purchased from supermarkets or the franchised store in Hong Kong. Details of these samples are listed in Table 1.

2.2. Apparatus

All experiments were carried out on a P/ACE MDQ electrophoresis system equipped with a photodiode array detector (Beckman Instruments, Fullerton, CA, USA) and a 60.2 cm (50 cm from the inlet to detector) × 50 μm I.D. fused-silica capillary tube (Beckman Instruments).

2.3. Electrophoretic procedure

The capillary tube was conditioned prior to its daily use by flushing with 0.1 M NaOH for 5 min, followed by water for another 5 min and finally with the buffer for 5 min. The running buffer was 20 mM borax with 3 mM β-cyclodextrin (without pH adjustment, pH = 9.4). The separation voltage was 25 kV, and separation temperature was 25 °C. Samples were injected under a pressure of 0.5 psi for 10 s. Electropherograms were recorded at 214 nm. The capillary was flushed between two separations with water (2 min), 0.1 M NaOH (1 min), water (2 min) and fresh buffer (2 min). The capillary tube was rinsed with 0.1 M NaOH for 30 min then with water for 30 min everyday after use.

2.4. Preparation of sample

About 10 g of turtle jelly was accurately weighed into a 50 ml centrifugal tube and mashed. The sample was extracted with ethyl acetate three times using a vortex mixer to agitate the mixture. The mixture was centrifuged at 5000 rpm for 5 min, then ethyl acetate was collected in a 25 ml volumetric flask and made up to the mark. The solution was centrifuged at 10,000 rpm for 5 min and then used as the sample solution. Before injection, 200 μl of p-courmaric acid (100 μg ml^{-1}), used as an internal standard, was added to 800 μl of sample solution.

For *RSG* concentrated extract, 0.1 g sample was accurately weighed, then 5 ml water was added and mixed with the vortex mixer. Finally, 20 ml methanol was added and thoroughly mixed again. The solution was centrifuged at 10,000 rpm for 5 min. Then, the supernatant was used as the sample solution. Before injection, 200 μl of p-courmaric acid (100 μg ml^{-1}), used as internal standard, was added to 800 μl of sample solution.

Table 1
Details of collected samples of turtle jelly.

Brand	Sample No.	Product Name	Origin	Ingredients
Hoi Tin Tong (fresh herbal jelly series)	S01	Fresh herbal jelly	Hong Kong	NA[a]
	S02	Pearl turtle jelly		NA[a]
	S03	Gui Ling Gow		NA[a]
	S04	Fu Ling Gao		NA[a]
Hoi Tin Tong (freshness retained jelly series)	S05	Fresh herbal jelly	Hong Kong	Fresh turtle shell, Fresh *RSG*, *Mesona chinensis* Benth., Fructus Alpiniae Oxyphyllae, Rhizoma Dioscoreae, Fresh Rhizoma Phragmitis
	S06	Pearl turtle jelly		Turtle shell, *RSG*, *Mesona chinensis* Benth., Nux Prinsepiae, Pearl, Folium Eucommiae, Radix Morindae, Herba cistanches, Padix Rehmanniae Praeparata, Rhizoma Polygonati, Radix Achyranthis Bidentatae
	S07	Fu Ling Gao		Fresh *RSG*, *Spina gleditsiae*, Radix Paeoniae Rubra, Fructus Aurantii, Radix Et Rhizoma Glycyrrhizae, Rhizoma Alismatis, Pericarpium Citri Reticulatae Viride, Herba Plantaginis, Radix Scutellariae
	S08	Herbal jelly with Ginseng		Fresh turtle shell, Fresh *RSG*, Ginseng, Fructus Arctii, Flos Lonicerae Japonicae, Radix Rehmanniae, Radix Ophiopogonis
Hungfooktong	S09	Herbal turtle jelly	Hong Kong	Turtle shell, *RSG*, Radix Rehmanniae, Flos Lonicerae japonicae, Fructus Aurantii immaturus, Herba Schizonepetae, Rhizoma Atractylodis, Cortex Dictamni, Radix Et Rhizoma Glycyrrhizae, *Mesona chinensis* Benth.
	S10	Turtle jelly with Lingzhi		Lingzhi, Turtle shell, *RSG*, Radix Rehmanniae, Herba Schizonepetae, Fructus Forsythiae, Rhizoma Phragmitis, Herba Abri, Radix Et Rhizoma Glycyrrhizae, *Mesona chinensis* Benth.
Healthworks	S11	Herbal turtle jelly	Hong Kong	NA[a]
	S12	Turtle jelly with Ginseng		NA[a]
	S13	Turtle jelly with Lingzhi		NA[a]
Guang Jian Tang	S14	Turtle jelly	China	Turtle shell, Flos Lonicerae Japonicae, *RSG*, Herba Taraxaci, Radix Angelicae Dahuricae, Fructus Gardeniae, Flos Sophorae, Radix Et Rhizoma Glycyrrhizae, Mesona chinensis Benth.
Shuang Qian	S15	Turtle jelly	China	Turtle shell, Flos Lonicerae Japonicae, *RSG*, Folium Mori, Herba Taraxaci, Radix Et Rhizoma Glycyrrhizae, Mesona chinensis Benth.
KungWoTong	S16	Turtle jelly	Hong Kong	NA[a]
Yeung Woo Tong	S17	Turtle jelly	Hong Kong	NA[a]
Hui Lau Shan	S18	Turtle jelly	Hong Kong	NA[a]

[a] NA = not available.

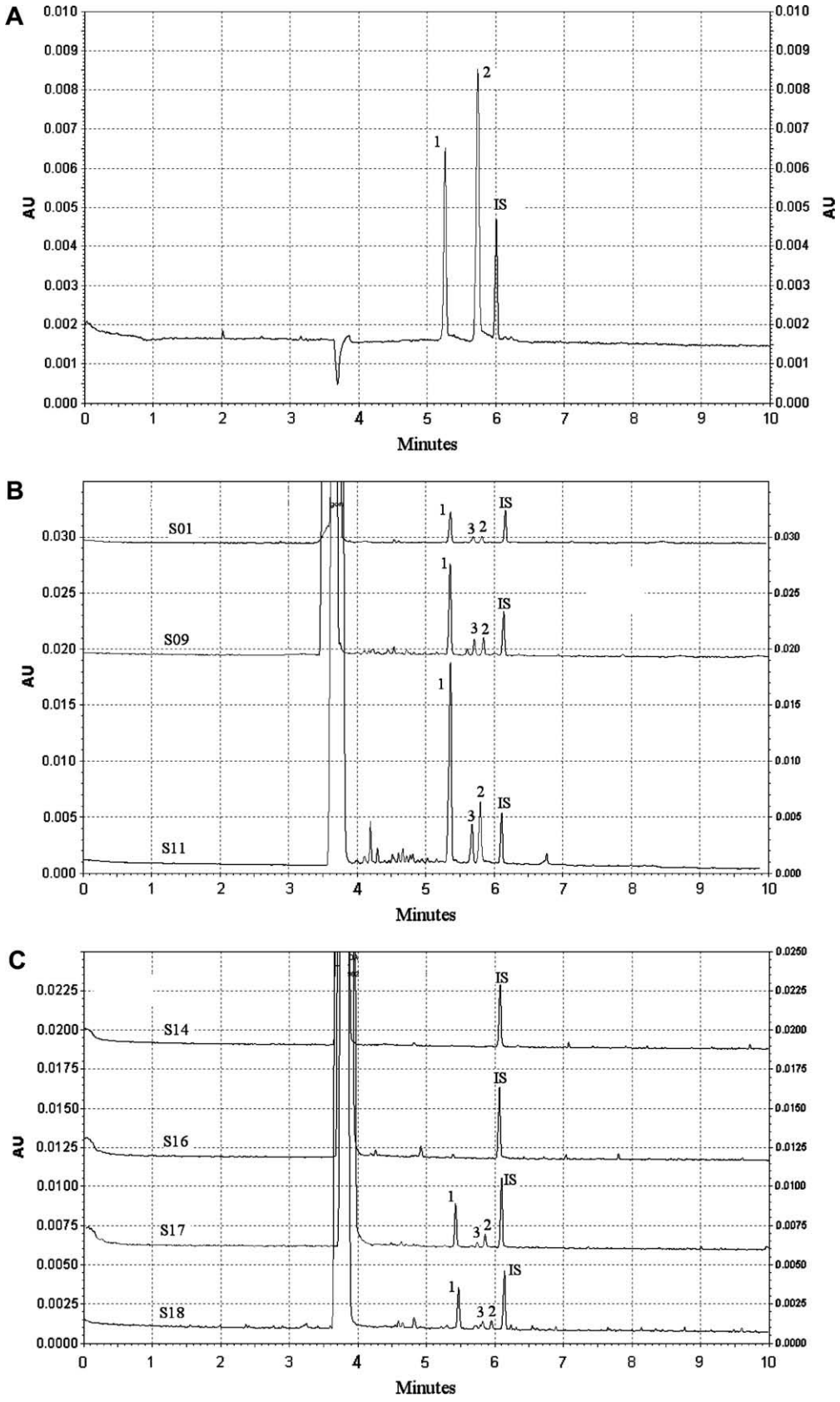

Fig. 1. Electropherograms of standards (A) and turtle jelly samples (B and C) under optimal separation conditions. Peaks: 1 = astilbin; 2 = taxifolin; IS: internal standard (p-coumaric acid).

3. Results and discussion

3.1. Method development

Capillary electrophoresis, an analytical technique which appeared in the early 1980s, is a separation technique that using high voltage to separate molecules based on differences in charge and molecule weight ratio. Because of its high separation performance, short analytical time, small quantity of sample required and environmental friendliness, the technique has attained more and more attention in food and pharmaceutical analysis (Castaneda, Rodriguez-Flores, & Rios, 2005; Cheung & Zhang, 2008; Garcia-Canas & Cifuentes, 2008). In the present study, the optimal separation was carried out with running buffer of 20 mM borax with 3 mM β-cyclodextrin (without pH adjustment, pH 9.4), separation voltage of 25 kV and temperature of 25 °C. The detection wavelength was 214 nm. Fig. 1A shows the electropherogram of standards under optimal separation conditions. As can be seen, the separation can be achieved within 7 min.

Because of the small sample injection volumes in CE (typically 5–50 nl), it is difficult to be precise between injections. Hence, an internal standard (IS) is always used to overcome this problem. Using p-coumaric acid (20 μg/ml) as the internal standard, the calibration curves of astilbin and taxifolin were obtained. The linear regression equations and correlation coefficients were: astilbin: $Y = 0.03786X - 0.07$ ($r = 0.9986$); taxifolin: $Y = 0.0700X + 0.03$ ($r = 0.9996$), where Y was the peak area ratio of analytes and p-coumaric acid, X was the concentration of analytes (0–100 μg/ml).

To validate the method, the relative standard deviation (RSD) of retention time (t_R) and peak area ratios (analyte peak area/internal standard peak area) of the standards at 20 μg/ml was investigated using the results from six injections. The results showed that the RSD of t_R and peak area ratios of astilbin and taxifolin were 0.62% and 2.17%, 0.87% and 3.62%, respectively. Accuracy of the assay was checked by adding 20 μg mL^{-1} of standards to the tested samples. Corrected peak area of each standard in the sample after spiking was compared with the sample alone plus the amount spiked. The result demonstrated that the recoveries of astilbin and taxifolin were 96.4% and 101.2%, respectively. These parameters indicate that the CE method is reliable for quantitative analysis of astilbin and taxifolin in the sample.

3.2. Turtle jelly sample analysis

The optimum conditions were applied for the separation and determination of astilbin and taxifolin in turtle jelly samples.

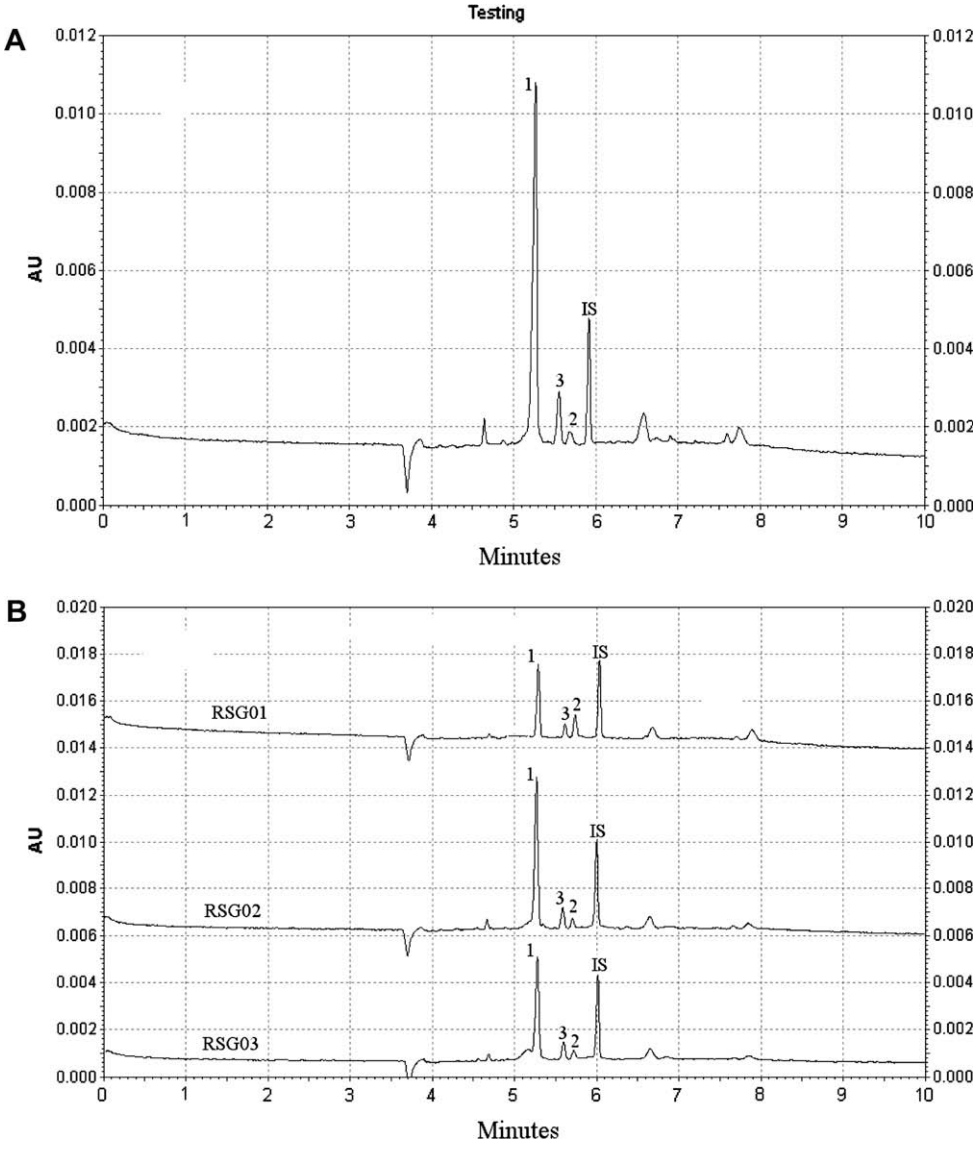

Fig. 2. Electropherograms of *RSG* (A) and its concentrated extract products (B). Peaks: 1 = astilbin; 2 = taxifolin; IS: internal standard (p-coumaric acid).

Table 2
Content of astilbin and taxifolin in different turtle jelly samples ($\mu g\ g^{-1}$).[a]

Sample No.	Astilbin	Taxifolin	Sample No.	Astilbin	Taxifolin
S01	85.49 ± 8.14	9.54 ± 0.89	S10	81.46 ± 7.67	11.40 ± 1.01
S02	23.76 ± 1.76	1.56 ± 0.34	S11	368.73 ± 21.37	59.65 ± 3.91
S03	66.85 ± 4.31	9.33 ± 1.05	S12	310.14 ± 17.65	54.36 ± 2.48
S04	44.21 ± 4.55	4.81 ± 0.81	S13	315.92 ± 15.98	75.72 ± 3.69
S05	21.26 ± 2.81	1.23 ± 0.67	S14	ND[b]	ND[b]
S06	36.02 ± 1.98	3.85 ± 0.45	S15	ND[b]	ND[b]
S07	57.38 ± 4.06	6.72 ± 1.07	S16	ND[b]	ND[b]
S08	149.47 ± 11.24	21.50 ± 1.65	S17	50.55 ± 3.71	5.02 ± 0.79
S09	171.46 ± 13.67	22.50 ± 1.21	S18	48.02 ± 4.52	7.65 ± 1.13

[a] Values are expressed as means ± S.D. of triplicate measurements.
[b] ND = not detected.

Representative electropherograms of some samples were shown in Fig. 1B and C. The peaks were identified by comparing their UV spectra and migration times with that of standards, and also by spiking the sample solution with standards. Compared with the standards, the migration times of astilbin and taxifolin in samples were slightly delayed. This may be because different solvents were used for the samples (ethyl acetate) and for standard solutions (methanol). However, the small migration time shift has no effect on peak identification, because we also have IS peak and the UV spectra of each analyte. Besides, we also spiked the sample with standards.

Comparing the electropherograms of different turtle jelly samples, three main peaks (1–3) can be found in most samples except for S15 and S16 (Fig. 1B and C). Peak 1, the biggest peak in all samples, was identified as astilbin and peak 3 was taxifolin. Although the various turtle jelly samples contained many other herbs as shown on their labels, it can be found that the three main peaks in jelly were all from *RSG* by comparing their electropherograms (Fig. 2A). Our results show that for many brands of turtle jelly in Hong Kong, *RSG* was the main ingredient. However, we could not find any peak in the electropherograms of S14 and S15, although on their labels it indicated that *RSG* was used.

The calculated contents of astilbin and taxifolin in all turtle jelly samples are listed in Table 2. As shown, the content of astilbin and taxifolin in different brands of turtle jelly were remarkably different. The content of astilbin in Healthworks brand were more than 300 μg/g, while in Hoi-Tin-Tong brand samples there was <100 μg/g. In contrast, some samples contained no astilbin and taxifolin at all (S14, S15 and S16). As shown by our previous work, astilbin was the key constituent in *RSG* and has good solubility in boiling water (Zhang et al., 2009), so it can be extracted from *RSG* by the decoction procedure and is found in turtle jelly. A pack of turtle jelly is about 250 g, so people will ingest high amount of astilbin and taxifolin by consuming a whole pack of this functional food. Using S11 as an example, the intakes of astilbin and taxifolin are about 92 and 15 mg, respectively, by consuming a pack of turtle jelly. Because astilbin and taxifolin have many bioactivities as mentioned in the Introduction, it can be concluded that the consumption of turtle jelly will lead to its consequent health benefits.

Table 3
Content of astilbin and taxifolin in herbal turtle jelly of Hungfooktong produced on different dates ($\mu g\ g^{-1}$).[a]

Sample.	Production date	Astilbin	Taxifolin
Herbal turtle jelly of Hungfooktong	2009.02.03	171.46 ± 10.67	22.50 ± 1.21
	2009.02.13	164.99 ± 12.13	17.06 ± 1.56
	2009.02.16	126.66 ± 8.98	13.72 ± 1.17
	2009.02.20	126.39 ± 9.84	14.16 ± 0.89
	2009.02.23	192.56 ± 14.21	19.64 ± 2.11

[a] Values are expressed as means ± S.D. of triplicate measurements.

Table 4
Content of astilbin and taxifolin in *RSG* concentrated extracts ($mg\ g^{-1}$).[a]

Sample No.	Brand	Batch No.	Astilbin	Taxifolin
RSG01	Sheng Chang Pharmaceutical CO., Ltd.	E410304	8.76 ± 0.67	1.48 ± 0.17
RSG 02	Kaiser Pharmaceutical CO., Ltd.	403451	15.78 ± 0.81	0.61 ± 0.07
RSG 03	Sun Ten Pharmaceutical CO., Ltd.	183305	12.90 ± 0.89	0.59 ± 0.08

[a] Values are expressed as means ± S.D. of triplicate measurements.

3.3. Quality consistency of turtle jelly product

The contents of astilbin and taxifolin in Herbal Turtle Jelly manufactured by Hongfooktong on different days were determined to evaluate quality consistency. As shown in Table 3, astilbin and taxifolin contents varied with the time of manufacture. The RSD of astilbin and taxifolin contents for the five batches were 18.6% and 21.3%, respectively. As the content of astilbin was significantly different in *RSG* cultivated in different geographical areas (Zhang et al., 2009), turtle jelly producers need quantitative analytical methods to be sure of the quality consistency of their products.

3.4. RSG concentrated extracts analysis

Three *RSG* concentrated extracts from Taiwan were also analysed by the present method. Fig. 2B shows the electropherograms of these products. As can be seen, their chemical profile was exactly the same with *RSG* as shown in Fig. 2A. However, the content of bioactive components was remarkably different between brands. As shown in Table 4, astilbin content in *RSG*02 was 15.78 mg/g, which is about twice the amount of *RSG*01. However, the taxifolin content in *RSG*01 was higher than that in the other two. These results may be due to the different materials and manufacturing processes used by different producers, which in turn, reflects the different compositions content in their products. Thus, the present method could be used for quality control of the products.

4. Conclusion

A CE method was successfully developed for the determination of astilbin and taxifolin in turtle jelly and *RSG* concentrated extract samples. The method is simple, fast and gives satisfactory results. Eighteen turtle jelly samples produced in mainland China and in

Hong Kong were collected for analysis. The results show that the three main peaks in turtle jelly were all from *RSG*, and astilbin was the key bioactive constituent. The contents of astilbin and taxifolin in different brands of turtle jelly were distinctly different; some brands did not even contain these compounds. Nevertheless, if astilbin and taxifolin show bioactivities, a measurable intake of these compounds may be conducive to good health. Thus, the present method is good for the quality control and the biological study of turtle jelly.

Acknowledgement

The authors thank the School of Graduate Studies of the City University of Hong Kong for a PhD studentship to Zhang Qing-Feng.

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