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Xiuchun Guo, Zhaoyang Xia, Miaomiao Song, Changqin Li, Jinmei Wang, Wenyi Kang*

Dynamic Changes of Secondary Metabolites and Antioxidant Activity of *Ligustrum lucidum* During Fruit Growth

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Abstract: There are a number of secondary metabolites having medicinal values in *Ligustri Lucidi Fructus*. In this study, the target analytes salidroside, ligustroflavone, specnuezhenide, oleuropein, oleanolic acid and ursolic acid were chosen, aiming to establish a method to investigate the content of six compounds during eight growth stages of *Ligustri Lucidi Fructus*. Even though the results indicated that the contents of six compounds in different growth periods reached their maximum value, they displayed a downward trend. The antioxidant activity of the analyzed samples also decreased along with the growth period. The relationship between the content of six secondary metabolites and the activity has been elucidated. Hence, this research provides a theoretical basis for guiding efficient use of *Ligustri Lucidi Fructus*.

Keywords: *Ligustri Lucidi Fructus*, Dynamic changes, Antioxidative effects, Sepctrum-efficacy

1 Introduction

Ligustri Lucidi Fructus (also named Shen Nong in Chinese) is a traditional Chinese medicine and is frequently used for clinical treatments of deficiencies of liver-yin and kidney-yin, vertigo, tinnitus, soreness and weakness of waist and knees, prematurely graying hair, blurred vision, inner heat of diabetes, osteopyrexia and fever [1]. *Ligustri Lucidi Fructus* has been discovered 2000 years ago and is used to support a healthy energy. Pharmacological researches have shown that *Ligustri Lucidi Fructus* possesses anti-inflammatory, anti-tumor and hypoglycemic action [2]. Meanwhile, it also enhances humoral immunity, cellular immunity and nonspecific immunity [3,4]. Phytochemical researches have shown that the genus contains mainly pentacyclictriterpenoid, iridoid and phenylethanoid glycosides [5,6]. Many studies have indicated that some secondary metabolites of *Ligustri Lucidi Fructus*, such as salidrosideis, oleuropein, ligustroflavone, and specnuezhenide, have different physiological activities [7,8,9,10,11]. However, *Ligustri Lucidi Fructus* also contains large amounts of heavy metals that will affect the safety in clinical application [12]. It has been reported that *Ligustrum lucidum* Ait is a kind of evergreen that can be used as a local environmental assessment indicator [13].

To date, the researches on *Ligustri Lucidi Fructus* have been mainly focused on chemical composition, pharmacological action, processing and quality control (QC) methods. The main chemical components of *Ligustri Lucidi Fructus* are terpenoids, flavonoids, phenethyl alcohol glycosides, volatile oils and phospholipids. It is reported that its main pharmacological effects include hepatoprotective, immune regulation, anti-atherosclerotic, anti-inflammatory and anti-aging. The main processing methods of *Ligustri Lucidi Fructus* are steaming or stewing with wine or vinegar, and stir-frying with salt-water [14,15]. It is also known that chemical compositions and pharmacological effects are influenced by the processing methods. Thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) have been adopted as the main QC methods [16,17,18,19]. However, few studies have been conducted on the temporal changes of a variety of components in *Ligustri Lucidi Fructus* [20]. Thus, we analyzed the temporal dynamic changes of six components of *Ligustri Lucidi Fructus* with HPLC and established the spectrum-effect relationship between dynamic changes and pharmacological activity.

***Corresponding author: Wenyi Kang:** Institute of Chinese Medical material, Henan University, Kaifeng 475004, China; Kaifeng Key Laboratory of Functional Components in Health Food, Kaifeng 475004, China, E-mail: kangweny@hotmail.com
Xiuchun Guo, Zhaoyang Xia, Miaomiao Song, Changqin Li, Jinmei Wang: Institute of Chinese Medical material, Henan University, Kaifeng 475004, China
Xiuchun Guo, Changqin Li, Jinmei Wang: Kaifeng Key Laboratory of Functional Components in Health Food, Kaifeng 475004, China

2 Experimental Procedure

2.1 Reagents and chemicals

Methanol and ethanol were chromatographic grade. Nitric acid and phosphoric acid were analytical grade. The ultrapure water was purchased from Hangzhou WahahaBaili Food CO. Ltd, (Zhejiang, China) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Tokyo Chemical Industry Co, Ltd (Japan). 2.2′-Azino-bis(3-ethylbenzo-thiazoline-6-sulfoncicacid)(ABTS) were obtained from Fluka (Japan). 2,4,6-Tri(2-pyridinyl)-1,3,5-triazine(TPTZ), butylatedhydroxytoluene (BHT), butyl-p-hydroxyanisole (BHA) and propyl gallic acid (PG) were obtained from Sigma (St. Louis, MS, USA). 6-hydroloxy-2,5,7,8-tetramethy-chroman-2-carboxylicacid (Trolox) was obtained from Aldrich Chemical CO, Ltd.

2.2 Apparatus

An LC-20AT Shimadzu high performance liquid chromatography system (Kyoto, Japan), was equipped with a degasser, a quaternary gradient low pressure pump, a CTO-20A column oven, an SPD-M20AUV-detector and an SIL-20A auto sampler. A KQ-500DB ultrasonic cleaner (Jiangsu Kunshan Ultrasonic Instrument Co, Ltd. Jiangsu, China) and 600Y plant sample pulverizer were obtained from BoaoHardwareFactory (Yongkang, Zhejiang, China). The sample sieves were obtained from Sieve Factory (Five Four instrument, Shangyu, Zhejiang, China); TGL-16gR super-centrifuge was obtained from An Ting Factory (Shanghai, China); AB135-S 1/10 million electronic balance was purchased from Mettler Toledo Instruments Co,Ltd. (Shanghai, China); Multiskan GO microplate reader was purchased from Thermo Fisher Scientific Co ,Ltd.(Waltham, MA, USA).

2.3 Plant materials and sample preparation

2.3.1 Plant materials

Ligustri Lucidi Fructus, identified by Professor Changqin Li, were collected from September 13th, 2015 to December 30th, 2015 in the campus of Henan University (Kaifeng, Henan, China). Every eight samples were pooled once every 15 days. An voucher specimen was deposited in the Institute of Traditional Chinese Medicine, Henan University. The samples were dried and shaded at room temperature. The dried plant materials were pulverized and then passed successively through 10, 24, 50, 70 and 90-mesh sieves.

2.3.2 Sample preparation for HPLC

The sample powder (20 mg) was passed through over 90-mesh and placed in a 1.5 mL of centrifuge tube; 1 mL of methanol was added and followed by ultrasonic extraction for 30 min. The extract was centrifuged at 3000 rpm for 5 min. The supernatant was saved and passed through a 0.22 μm microporous membrane. The filtrate was obtained and used as the sample solution.

Preparation of standard solution: salidroside, ligustroflavone, specnuezhenide, oleuropein, oleanolic acid and ursolic acid were used as standard reference substances. They were precisely weighed as 4.12, 4.91, 4.92, 4.82, 2.71 and 2.80 mg and placed into a 10 mL of volumetric flask, dissolved and diluted with methanol to the final volume. The mixed solution was filtered through a 0.22 μm microporous membrane prior to an injection into HPLC system.

2.3.3 Chromatographic conditions

The chromatographic conditions for salidroside, ligustroflavone, specnuezhenide, oleuropein, oleanolic acid and ursolic acid were followed as: the chromatography was carried out on an Agilent Eclipse XDB-C18 column (4.6 ×250 mm, 5 μm) at a column temperature of 25°C. The mobile phase was consisted of methanol (A) and 0.1%phosphoric acid water (B). The gradient elution steps were set as follows: 0-15min, 20-45%A, 80-55%B; 15-45min, 45%A, 55%B; 45-60min, 45-85%A, 55-15%B; and 60-120min, 85%A, 15%B) at a flow rate of 0.6 mL/min. The UV detection wavelength was set at 210 nm with a sample injection volume of 10 μL.

2.3.4 Antioxidant activity in vitro

2.3.4.1 Preparation of the sample solution

According to the optimized conditions, methanol and sample solutions were prepared and concentrated to 1 mL, respectively.

2.3.4.2 DPPH assay

The sample was diluted into a range of concentrations with methanol. The sample solution (10 μL), methanol (10 μL) and 200 μM DPPH-methanol solution (175 μL) were added to a 96 microplate and mixed well, respectively. The solution was measured at 515 nm using a Multiskan GO microplate reader after incubation for 20 min in a dark place using PG, BHA and BHT as positive controls. All experiments were performed in triplicate. The percentage of radical scavenging rate was calculated using formula 1. The IC_{50} value was calculated from the concentration-effect linear regression curve [21].

DPPH radical scavenging rate (%) =

$$= [(A_0\text{-}A_1)/A_0] \times 100\% \quad (1)$$

Where A_0 is the absorbance of the DPPH itself, A_1 is the absorbance of sample and positive control. IC_{50} represents the concentration of a sample in μg/mL that inhibits DPPH radicals by 50%.

2.3.4.3 ABTS assay

According to the method described in the literature [22], ABTS radical working solution was prepared. The sample solution was diluted into a range of concentrations with methanol. 10 μL of sample solution and 200 μL of ABTS solution were added to a 96 microplate and mixed well. The solution was measured at 405 nm after incubation for 20 min in a dark place. All the experiments were performed in triplicate. The percentage of radical scavenging rate and IC_{50} value were calculated.

2.3.4.4 FRAP assay

According to the method described in literature [23], the TPTZ working solution was prepared. Then, the sample solution was diluted into a range of concentrations with methanol. 10 μL of sample solution and 200 μL of TPTZ solution were added to a 96 microplate and mixed well. The solution was measured at 593 nm after incubation for 20 min at 37°C. All the reactions were carried out with three replications. The ability of reducing Fe^{3+} of a sample was calculated. The results were expressed as μmol Trolox equivalents (TE)/g sample. The standard curve was linear when the concentrations of Troloxranged between 50 and 1600 μM. A further dilution of the sample was needed as the measured FRAP value exceeded the linear range of the standard curve.

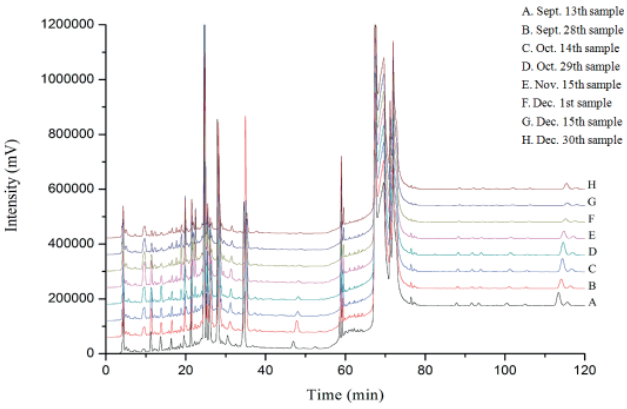

Figure 1: Chromatogram of eight growth periods of *Ligustri Lucidi Fructus*.

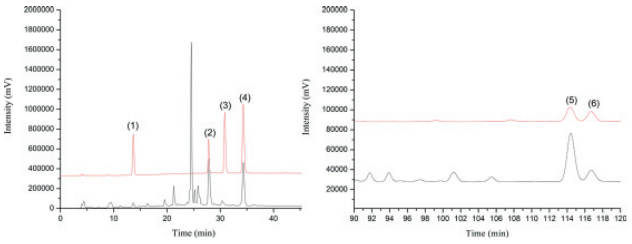

Figure 2: HPLC chromatograms of standard reference substances (red) and a sample. 1, Salidroside; 2, Specnuezhenide; 3, Ligustroflavone; 4, Oleuropein; 5, Oleanolic acid; and 6, Ursolic acid

Ethical approval: The conducted research is not related to either human or animals use.

3 Results and Discussion

The optimal conditions were determined as following: the ratio of sample (filtered through over 90-mesh) to extraction solvent (100% methanol), 1:50; ultrasonic time, 30 minutes; and centrifugal speed, 3000 rpm. Under these optimal conditions, the extraction rates of six target analytes were the highest. Using the method described in section 2.3, the sharpest peak shapes and the best peak symmetry were obtained. Figure 1 was the HPLC chromatogram of the extract of *Ligustri Lucidi Fructus* in eight different periods, which showed the changes of main components in *Ligustri Lucidi Fructus* in eight different periods. Figure 2 was the HPLC chromatogram of six standard substances (red) and one sample solution which was collected on December 15th, 2015. It showed the peak time of six standard substances and the comparison between the sample and the standard substances.

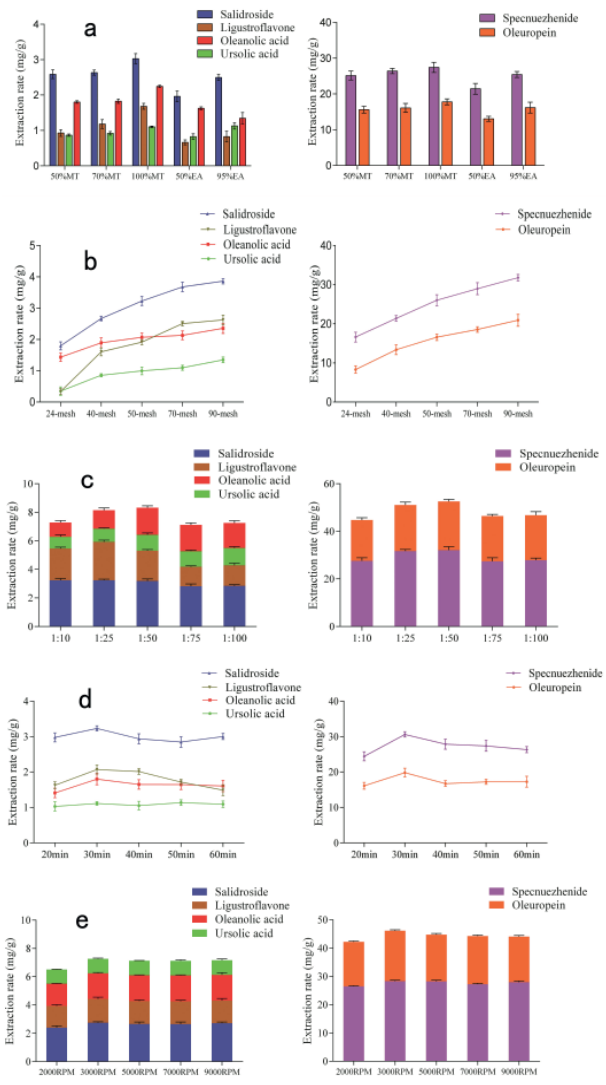

Figure 3: Optimization of extraction conditions on extraction efficiencies of six compounds.
a: extraction solvents; b: sample mesh numbers; c: solid-to-liquid ratios; d: ultrasonic times; f: centrifugal speeds

3.1 Optimization of Extraction Conditions

3.1.1 Effect of Extraction Solvents

In this experiment, 50%, 70% and 100% methanol, and 50% and 95% ethanol in water were selected as extraction solvents based on a comprehensive consideration of the physicochemical properties of salidroside, ligustroflavone, specnuezhenide, oleuropein, oleanolic acid and ursolic acid. The extraction efficiencies of 50% methanol, 70% methanol, 100% methanol, 50% ethanol and 95% ethanol were investigated. The results were shown in Figure 3a. Pure methanol had the optimal effect among the five extraction solvents tested. Thus, 100% methanol was adopted in the subsequent work.

3.1.2 Effect of Sample mesh Numbers

In our study, extraction efficiencies of six compounds displayed significant difference caused by the size of the crushed particles. According to the experimental methods described in the method section, 100% methanol was selected as extraction solvent and the extraction efficiencies of 10, 24, 50, 70 and 90-mesh sieves were investigated while the other conditions remained unchanged. The results were shown in Figure 3b. The samples of small particles had a larger contact surface area with the extracting solution and more analytes of the secondary metabolites were obtained. The 90-mesh sieve had the best effect among the 10-, 24-, 50-, 70- and 90-mesh sieves. Thus, the sample passed through over 90-meshwas adopted.

3.1.3 Effect of Solid-to-liquid Ratio

In this study, the solid-to-liquid ratio was another leading factor influencing the extraction efficiencies. The ratios of sample to 100% methanol were set as 1:10, 1:25, 1:50, 1:75, and 1:100. The results were shown in Figure 3c. The sample was extracted completely with quantitative extraction solution, extraction rate of the target was close to the data whose solid-to-liquid ratios were 1:25 and 1:50. Under these conditions, the secondary metabolites were completely extracted. According to the less sample consumption, solid-to-liquid ratio of 1:50 was adopted.

3.1.4 Effect of ultrasonic time

Different lengths of ultrasonic time had an effect on extraction yields of six compounds. The extraction efficiencies of ultrasonic times of 20, 30, 40, 50 and 60 min were investigated. The results were shown in Figure 3d. The extraction rate of six compounds was parabolic with ultrasound time extended with a peak value after 30 minutes. Thus, ultrasonic time for 30 minutes was adopted.

Table 1: Regression equations and linear ranges for six compounds.

Compound	Regression equations [a]	*r*	Linear range (μg)	LOD (ng)	LOQ (ng)
Salidroside	Y=1652254.5X-15206.75	0.9999	0.205 ~ 6.15	0.21	0.70
Ligustroflavone	Y=5025150.7X+23334.85	0.9999	0.098 ~ 7.35	0.74	2.46
Specnuezhenide	Y=1671639.9X-10044.08	0.9999	0.98 ~14.7	0.32	1.07
Oleuropein	Y=3263726.9X+189199.81	0.9998	0.096 ~ 7.2	0.89	2.96
Oleanolic acid	Y=885631.5X-61565.86	0.9999	0.135 ~ 2.7	0.17	0.57
Ursolic acid	Y=790622.8X-46205.18	0.9997	0.14 ~2.8	0.13	0.43

a. *Y*: Peak area; *X*: injection volume (μL).

3.1.5 Effect of Centrifugal Speed

Under the above optimization conditions, the centrifugal speed was chosen. It affected the time of contact between the sample and extraction solution. The extraction efficiencies of centrifugal speeds at 2000, 3000, 5000, 7000 and 9000 rpm were investigated. The results were shown in Figure 3e. The extraction efficiency at centrifugal speed of 3000 rpm was maximal.

3.2 Linear relationship

The standard solution with the volumes of 0.2, 1, 2, 5, 10, 15 and 20 μL was accurately injected to chromatographic instrument for the construction of calibration curves, respectively. Then, the calibration curves were constructed by plotting the peak areas versus the injection volume of each compound. The regression equations, *r* values, linear range, limit of detection (LOD) and limit of quantitation (LOQ) were presented in Table 1. The *r* values were in the range from 0.9997 to 0.9999, indicating that the methods have quite good linearity.

3.2.1 Determination of the Contents of Six Components in samples

The samples were collected under verification tests on December 15th, 2015, under the following optimal conditions: the samples were filtered through a 90-mesh sieve; the ratio of samples to extraction solvent (100% methanol), 1:50; ultrasonic extraction time; 30 min; and centrifugal speed, 3000 rpm. The concentrations of salidroside, ligustroflavone, specnuezhenide, oleuropein, oleanolic acid and ursolic acid were determine to be 0.412, 0.491, 0.492, 0.482, 0.271 and 0.28 mg/mL, respectively.

3.2.2 Repeatability for HPLC

Six sample solutions were selected on the same time, and 10 μL of solutions were injected into chromatographic instrument for analysis. The relative standard deviations (RSD) of peak areas for the six compounds were 0.58, 0.43, 0.59, 0.26, 1.39 and 1.78%, respectively, which indicate that the analytical methods were reproduced well, determining the contents of six compounds of *Ligustri Lucidi Fructus*.

3.2.3 Precision Experiment for HPLC

The standard solution (10 μL) was accurately injected to chromatographic instrument, and analyzed in six replicates within one day for determining the precision of the developed assay. The RSDs of peak areas for the six compounds were 0.14, 0.15, 0.14, 0.21, 0.21 and 1.48%, respectively. The results indicate that the method was precise for quantitative analysis of salidroside, ligustroflavone, specnuezhenide, oleuropein, oleanolic acid and ursolic acid.

3.2.4 Stability for HPLC

The sample solutions were placed at room temperature and 10 μL of solution were injected into chromatographic instrument at 0, 4, 8, 12, 18 and 24 h, respectively. The RSDs

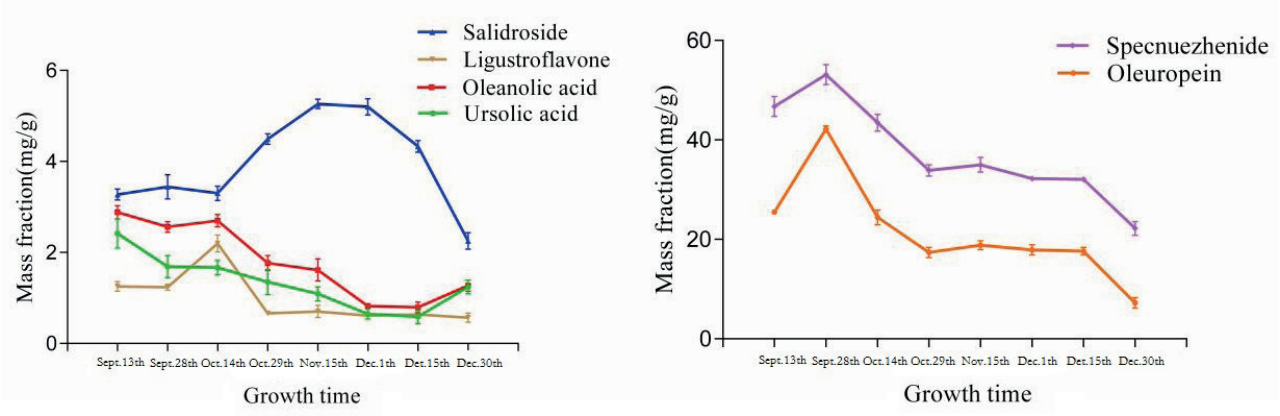

Figure 4: Temporal changes in the contents of six compounds in different growth periods.

of their peak areas were 1.10, 0.68, 0.50, 0.53, 0.83 and 1.26%, respectively. The results indicated that the sample solutions were basically stable at room temperature within 24 h.

3.2.5 Recovery for HPLC

The sample recovery was conducted as following: nine samples with known contents were divided into three groups. Then, six standard compounds at three different concentrations were added to the sample, respectively. All the recovery values of the analytes were calculated and they ranged from 99.96% to 102.54% and the RSDs were less than 1.83%. The results demonstrated that the methods were reasonable and feasible.

3.3 Temporal dynamic changes of six compounds

In Figure 4, there was a parabola relationship between six secondary metabolites and time, in which the concentrations of salidroside and ligustro flavones reached peak value at mid-October and mid-November, respectively. The concentrations of spenuezhenide and oleuropein reached the peak value at the end of September. The concentrations of oleanolic acid and ursolic acid were reduced until the end of December and the overall trend was reduced. Salidroside and specnuezhenide were reported to alleviate osteoporosis in the prevention of osteoporosis [24]. Oleuropein could activate pepsin inhibition of all other enzymes to reduce coronary heart disease and cancer [25]. Berberine hydrochloride could increase the ATP/AMP ratio to activate AMPK by inhibiting the mitochondrial functions. It has been shown that ligustro flavone has the same effect as berberine hydrochloride [26]. According to literature [27,28,29,30,31], oleanolic acid and ursolic acid were used to treat diabetes by stimulating and improving insulin, inhibiting the absorption of lipids, and promoting lipid metabolism. Therefore, in order to understand the temporal dynamic changes in their contents it is important to select the appropriate picking time. The research by Qiu et al. showed that the contents of secoiridoid glycosides in *Ligustri Lucidi Fructus* were changed significantly after processing and that salidroside was produced by specnuezhenide and G13 were hydrolyzed as steaming with wine [32]. Thus, the increase of salidroside was related to a reduction of secoiridoid glycosides between September 13th and December 1st. The contents of salidroside, oleuropein and specnuezhenide were decreased obviously after frost's descent. According to the research of Huang et al., light intensity, soil and temperature affected the contents of four alkaloids in catharanthusroseus [33]. It is suggested that decrease in temperature affected the secondary metabolism rates of plants, resulting in the decrease of the contents of secondary metabolites.

3.4 Dynamic changes of antioxidant activity of the different times of *Ligustrum lucidum Fructus in vitro*

More and more studies suggested that natural substances exhibited high bioactivity in scavenging reactive oxygen species (ROS) and could effectively reduce risks in some diseases, such as cardiovascular disease, nonalcoholic fatty liver disease and diabetes [34,35,36]. As shown in Table 2, the ability of antioxidation *in vitro* had become increasingly weak with the fruit ripening. According

Table 2: Antioxidant activity of different times of Ligustrum lucidum Fructus.

Sample	DPPH		ABTS		FRAP
	Radical scavenging rate(%)	IC_{50} (μg/mL)	Radical scavenging rate (%)	IC_{50} (μg/mL)	TEAC (μmol/g)
Sept.13th sample	95.21	148.06±1.51	98.35	29.17±2.09	513.22±5.75
Sept.28th sample	95.32	171.96±0.99	98.06	28.92±1.71	624.03±5.88
Oct.14th sample	95.47	197.85±22.61	97.45	24.66±2.56	405.82±4.26
Oct.29th sample	94.72	187.35±8.91	97.21	32.68±1.88	495.85±5.03
Nov.15th sample	94.75	181.76±4.06	97.84	42.21±2.13	451.21±6.74
Dec.1st sample	94.17	177.42±1.18	98.48	38.28±3.99	476.25±7.93
Dec.15thsample	94.54	232.96±0.37	97.59	31.85±0.43	552.80±7.43
Dec.30th sample	95.07	387.14±23.81	98.17	47.40±0.75	305.46±7.43
BHA[a]	74.04	14.31±1.63	99.95	2.56±0.05	5919.11±14.10
BHT[b]	72.06	11.41±0.39	99.66	6.56±0.13	589.20±59.20
PG[c]	93.23	5.42±0.12	95.85	0.72±0.05	9441.33±69.27

a. BHA served as the positive controls.
b. BHT served as the positive controls.
c. PG served as the positive controls.

to reference chromatogram by samples collected in September 13th, 2015, a good chromatographic peak was selected in the software named The Similarity Evaluation System For Chromatographic Fingerprint of TCM (2004 Editon A). Using multipoint algorithm based on least-mean-square method about the peak, 28 peaks were matched for research objects. The antioxidant activities of the *Ligustrm Lucidum Fructus* in different growth periods were used as *Y* axis and the characteristic chromatographic peak areas were quantified as *X* axis. The data were treated by zero-mean normalization in DPS7.05 and a mathematical model was established based on Partial Least Squares regression. Error square and R^2 were firstly increased and then decreased with the increasing latent factor. R^2 arrived at maximum when latent factor was six. Three regression equations were obtained as following.

$$Y_1 = 0.000000 + 0.044652X_1 + 0.081246X_2 - 0.214123X_3 + 0.050495x_4 - 0.027462X_5 - 0.109034X_6 + 0.003437X_7 + 0.172990X_8 - 0.179894X_9 - 0.024711X_{10} + 0.199526X_{11} + 0.167389X_{12} + 0.139870X_{13} + 0.075206X_{14} + 0.063206X_{15} + 0.012277X_{16} + 0.082461X_{17} - 0.025129X_{18} + 0.036047X_{19} + 0.050520X_{20} + 0.097218X_{21} + 0.059327X_{22} + 0.104195X_{23} - 0.137903X_{24} + 0.262917X_{25} - 0.157641X_{26} + 0.043910X_{27} + 0.127165X_{28}$$

$$Y_2 = 0.000000 + 0.201567X_1 + 0.229796X_2 - 0.104017X_3 + 0.115755X_4 + 0.025932X_5 - 0.000502X_6 - 0.154787X_7 + 0.019990X_8 + 0.041956X_9 - 0.002647X_{10} + 0.111504X_{11} + 0.082888X_{12} + 0.139451X_{13} - 0.026971X_{14} - 0.037631X_{15} + 0.012305X_{16} + 0.076421X_{17} - 0.094109X_{18} + 0.072660X_{19} + 0.018408X_{20} - 0.038313X_{21} + 0.068231X_{22} + 0.013541X_{23} - 0.092207X_{24} + 0.218654X_{25} - 0.060441X_{26} + 0.021014X_{27} + 0.066107X_{28}$$

$$Y_3 = -0.000000 + 0.248401X_1 + 0.183568X_2 - 0.176618X_3 + 0.109600X_4 - 0.042863X_5 - 0.121455X_6 - 0.086001X_7 + 0.056981X_8 - 0.101348X_9 - 0.097675X_{10} + 0.161334X_{11} + 0.180208X_{12} + 0.227099X_{13} + 0.051707X_{14} + 0.055479X_{15} - 0.026648X_{16} + 0.091593X_{17} - 0.063117X_{18} + 0.074231X_{19} - 0.000365X_{20} + 0.040706X_{21} + 0.033778X_{22} + 0.090182X_{23} - 0.251375X_{24} + 0.311467X_{25} - 0.236723X_{26} - 0.030904X_{27} + 0.066476X_{28}$$

where Y_1 was opposite number of IC_{50} value in DPPH, Y_2 was opposite number of IC_{50} value in ABTS, Y_3 was equivalent Trolox in FRAP, and X_i represented the peak area.

As shown in Figure 5, the higher the absolute value of *Y* was, the more remarkable the influence of corresponding *X* was [37]. If *Y* value was positive, it was positively correlated to antioxidant capacity. If *Y* value was negative, it was negatively correlated to antioxidant capacity. The results indicated that S2, S4, S8, S11, S12, S13, S14, S15, S16, S17, S19, S23, S25, S27, and S28 were positively correlated to opposite numbers of IC_{50} values in DPPH, which means that samples have strong capacity of scavenging DPPH free radicals with the peaks increased. S1, S3, S5, S6, S7, S9, S10, S18, S20, S21, S22, S24and S26 were negatively correlated to opposite number of IC_{50} values in DPPH. Samples had weak capacity of scavenging DPPH free radicals with the peaks increased. S1, S2, S4, S5, S6, S9, S10, S11, S12, S13, S14, S16,S17, S19, S22, S23, S25, S27 and S28 were positively correlated to opposite number of IC_{50} values in ABTS, indicated that sample had strong

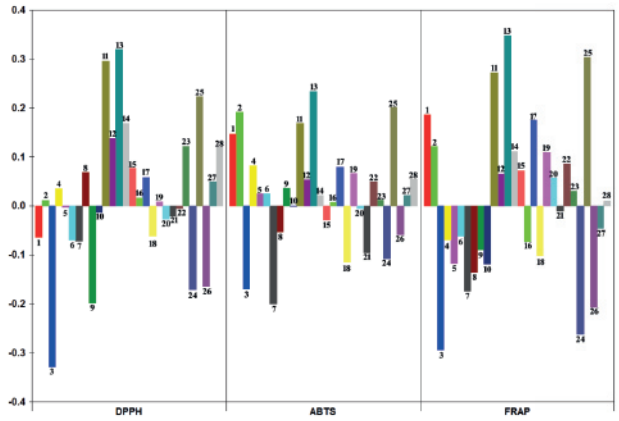

Figure 5: Standardization regression coefficients of three PLSR models of Ligustri Lucidi Fructus.

capacity of scavenging ABTS free radical with the peaks increased. S1, S3, S5, S6, S7, S9, S10, S18, S20, S21, S22, S24 and S26 were negatively correlated to opposite numbers of IC_{50} values in ABTS, indicated that sample had weak capacity for scavenging ABTS free radical with the peaks increased. S1, S2, S11, S12, S13, S14, S15, S17, S19, S20, S22, S23, S25 and S28 were positively correlated to equivalent Trolox in FRAP, indicated that sample had strong capacity of reduction of Fe^{3+} with the peaks increased. S3, S4, S5, S6, S7, S8, S9, S10, S16, S18, S24, S26 and S27 had the opposite effect. Salidroside, ligustroflavone, specnuezhenide, oleuropein, oleanolic acid and ursolic acid correspond to S11, S12, S23, S25, S27 and S28, respectively, by comparing the retention time of HPLC. The antioxidant capacity was decreased wby the development of fruits. Because *Ligustri Lucidi Fructus* attained their maturity in winter, we inferred that the decrease in temperature affected the generation of secondary metabolites. The decrease of six marker compounds directly affected the ability of an antioxidation *in vitro*.

4 Conclusions

Determination of the contents of six compounds in different developmental stages of *Ligustri Lucidi Fructus* led us to discover the changing patterns of secondary metabolites and their antioxidant activity in different growth periods of the fruit Based on these results, conclusion can be drawn that *Ligustri Lucidi Fructus* has antioxidant activity in the body. The results of this study are of both theoretical significance and practical value.

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