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Determination of catechins in green tea leaves by HPLC compared to spectrophotometry

Aim. To study the qualitative composition, the quantitative content of catechins in green tea leaves and compare the data obtained with those evaluated by spectrophotometry.

Materials and methods. Green tea leaves used for the analysis were collected in Anhui Province, China. The extract for the HPLC analysis was obtained by the maceration method with 60% ethanol twice in the *raw material* / *extractant* ratio of 1:20. In the case of the spectrophotometric analysis, green tea leaves were extracted with 70% ethanol twice by the maceration method in *the raw material* / *extractant* ratio of 1:20. The analysis of the extract from green tea leaves was performed by high performance liquid chromatography using a Prominence LC-20 Shimadzu chromatographic system (Japan) with a SPD-20AV spectrophotometric detector, an Agilent Technologies Microsorb-MV-150 column (reversed phase, C18 modified silica gel, length – 150 mm, diameter – 4.6 mm, particles size – 5 μm). Substances in the extract were identified by comparing the retention time and the spectral characteristics of the test substances with the same characteristics of the reference standards. Spectrophotometric measurements were carried out using a UV-1000 single beam spectrophotometer (China) with the pair of S90-309Q quartz square cells.

Results and discussion. Using high performance liquid chromatography 5 catechins were identified. Among them epigallocatechin-3-O-gallate (10.85%) predominated, while catechin (0.61%) had the lowest concentration. The total amount of catechins in green tea leaves was 30.56 and 24.79% by HPLC and spectrophotometry, respectively. The *F*- and *t*-tests showed that there was no significant difference between the results of HPLC and spectrophotometry.

Conclusions. The qualitative composition and the quantitative content of catechins have been determined in the extract from green tea leaves by high performance liquid chromatography and spectrophotometry. Both HPLC and spectrophotometric methods can be used to determine the total catechin content in green tea leaves. The high content of catechins makes the extract promising for further study and creation of new herbal medicinal products and dietary supplements. The results obtained will be used for standardization of green tea leaves and for future pharmacological research of its extract.

Key words: green tea; catechins; leaves; high performance liquid chromatography; spectrophotometry

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Визначення катехінів у листі зеленого чаю методом ВЕРХ у порівнянні з методом спектрофотометрії

Мета. Методом ВЕРХ вивчити якісний склад і кількісний вміст катехінів листя зеленого чаю та порівняти результати з даними, отриманими методом спектрофотометрії.

Матеріали та методи. Для аналізу використовували листя зеленого чаю, зібране в провінції Аньхой, Китай. Для ВЕРХ-аналізу екстракт отримували двічі методом мацерації 60% спиртом у співвідношенні *сировина* / *екстрагент* 1:20. У випадку спектрофотометричного аналізу листя зеленого чаю двічі екстрагували 70% спиртом методом мацерації у співвідношенні *сировина* / *екстрагент* 1:20. Аналіз витяжки листя зеленого чаю проводили методом високоефективної рідинної хроматографії за допомогою хроматографічної системи Prominence LC-20 Shimadzu (Японія) зі спектрофотометричним детектором SPD-20AV, колонка Agilent Technologies Microsorb-MV-150 (обернено-фазова, С18 модифікований силікагель, довжина 150 мм, діаметр 4,6 мм, розмір зерен сорбенту 5 мкм). Ідентифікацію речовин у витяжці проводили шляхом порівняння часу утримування і спектральних характеристик досліджуваних речовин з аналогічними характеристиками стандартів. Спектрофотометричні вимірювання виконували за допомогою однопроменевого спектрофотометра UV-1000 (Китай) з парою кварцових кювет S90-309Q.

Результати та їх обговорення. За допомогою високоефективної рідинної хроматографії у листі зеленого чаю було ідентифіковано 5 катехінів, з-поміж яких переважає епігалокатехін-3-О-галат (10,85%), а найменший вміст мав катехін (0,61%). Сумарний вміст катехінів у досліджуваній сировині склав 30,56% та 24,79% за методом ВЕРХ та спектрофотометрією відповідно. Розрахунки *F*- і *t*- продемонстрували, що немає суттєвої різниці між результатами ВЕРХ та спектрофотометрією.

Висновки. Якісний склад та кількісний вміст катехінів в екстракті листя зеленого чаю визначено за допомогою високоефективної рідинної хроматографії та спектрофотометрії. Для визначення загального вмісту катехінів у листі зеленого чаю можна використовувати як ВЕРХ, так і спектрофотометричний метод. Високий вміст катехінів в екстракті робить цю сировину перспективною для подальшого вивчення і створення нових фітопрепаратів та дієтичних добавок. Отримані результати будуть використані для стандартизації листя зеленого чаю та для подальших фармакологічних досліджень його екстракту.

Ключові слова: зелений чай; катехіни; листя; високоефективна рідинна хроматографія; спектрофотометрія

Introduction

Tea has been used as a traditional medicine in China for more than 1000 years. Today, tea is used as a beverage and as an ingredient in cosmetics because of its antiaging properties. There are different types of tea, for example, white, green, oolong, black and Pu-erh tea and all of them are being produced from *Camellia sinensis* [1].

The chemical composition of green tea leaves includes proteins, chlorophyll, minerals and trace elements, volatile compounds, amino and organic acids, lignins, alkaloids (caffeine, theophylline, and theobromine), and polyphenols [2]. The main quality parameter for tea is the content of catechins. *M. Naldi et al.* investigated the composition of tea catechins in different species of green tea leaves by UHPLC; as a result, they estimated that the content of epicatechin (EC) was 0.45–1.10%, epigallocatechin (EGC) – 1.64–4.39%, epicatechin gallate (ECG) – 1.45–1.73%, epigallocatechin gallate (EGCG) – 5.94–9.26% in dry leaves [3]. Catechins possess various pharmacological properties, including antibacterial [4], antihypertensive [5], antioxidative [6], antiarteriosclerotic [7], anticarcinogenic [8] and hypocholesterolemic effects [10]. Due to these beneficial effects of catechins on the human health there is high scientific interest to green tea.

Nowadays, analytical methods, such as ion chromatography [11], high performance liquid chromatography-ultraviolet detection (HPLC-UV) [12], capillary electrophoresis [13], photocolorimetry [14], UV-spectrophotometry [15] and thin-layer chromatography [16], are commonly applied to analyze catechins.

HPLC methods are precise, accurate, with good reproducibility, but the cost of analysis is quite high because of expansive instrumentation, reagents and expertise, whereas the spectrophotometric method is express, simple for the quantitative determination. However, it is not selective and requires the analytes present in the given sample to have a different UV-spectrum with a low overlap.

There are variety of products with a green tea extract as dietary supplements, cosmetology products, tinctures and drugs. Generally, the green tea extract is also used as an antioxidant compound. Moreover, the literature survey [17] shows that the green tea extract is applied in weight loss due to decreasing adsorption of fats as catechins inhibit lipase. In regard to a wide application of the green tea extract, the study of the qualitative composition and the quantitative content of catechins in green tea leaves is topical issue today.

By virtue of great relevance of green tea to modern life, the aim of the study was to determine the qualitative composition, the quantitative content of catechins in green tea leaves and compare the data obtained with those evaluated by spectrophotometry. The results of the catechin content obtained will be further used to create dietary supplements with the green tea extract.

Materials and methods

Standards of catechin, epigallocatechin-3-O-gallate, epicatechin, epigallocatechin, epicatechin gallate were obtained from Sigma-Aldrich. Methanol was of HPLC grade, and other chemicals were of analytical grade.

A Prominence LC-20 Shimadzu liquid chromatography system equipped with a Thermo Scientific Syncronis aQ C18 column (4.6 mm × 250 mm) was used for analyses. All determinations were undertaken at 40°C. Mobile phases included an aqueous solution of methanol (**A**) and 1.0% phosphoric acid solution (**B**). Gradients of 20–42% of **A** for 0–15 min, 42–43% of **A** for 15–25 min, 43–90% of **A** for 25–45 min, keeping 90% of **A** for 45–55 min, decreasing to 20% of **A** for 55–60 min, and keeping 20% of **A** for 60-70 min were used. The mobile phases were filtered (25 mm × 0.45 μm, Supelco Iso-Disc Filters PTFE 25-4) and degassed prior to use; the flow rate of 0.5 mL/min was maintained. The sample injection volume was 5 μL, and detection was carried out at 255, 286, 350 nm.

Spectral characteristics of the substances and similarity indices were used for identification and quantification of the substances analyzed in the green tea extract [18].

As the spectral characteristic of the substances (h) the peak heights of these substances on the chromatograms at wavelengths of 255, 286 and 350 nm divided by the peak height at a wavelength of 225 nm were used:

$$h_{255} = \frac{H_{255}}{H_{225}}, \; h_{286} = \frac{H_{286}}{H_{225}}, \; h_{350} = \frac{H_{350}}{H_{225}},$$

where: H_{225}, H_{255}, H_{286}, H_{350} – are the peak heights at 225, 255, 286 and 350 nm, respectively; h_{255}, h_{286}, h_{350} – are relative peak heights at 255, 286 and 350 nm, respectively.

Chromatographic peaks of analytes were identified by the following similarity indices, which were calculated between the test substance and the reference standard according to the formulas:

$$I_T = 1 - |T_{st} - T_u|$$

$$I_{255} = 1 - |h_{255_{st}} - h_{255_u}|$$

$$I_{286} = - |h_{286_{st}} - h_{286_u}|$$

$$I_{350} = 1 - |h_{350_{st}} - h_{350_u}|$$

where: I_T – is the similarity index of the retention time; T_{st} – is the retention time of the standard (min); T_u – is the retention time of the test substance (min); I_{255}, I_{286} and I_{350} – are the spectral similarity indices; h_{255st}, h_{286st} and h_{350st} – are the spectral characteristics of the standard; h_{255u}, h_{286u} and h_{350u} – are the spectral characteristics of the test substance.

The lowest of the three values of the similarity indices of spectral characteristics determines the degree of similarity (I_L) the substances and the standards for these characteristics. The higher value of I_L is, the more likely it is that the substance is more accurately identified. Substances, which degree of similarity with the catechin standard was not less than 0.7, and the peaks of these substances on the chromatogram were located in the range between the peak of catechin and the earliest peak of flavonoids, were identified as catechins. The quantification was made by the peak integration using the external standard method.

Preparation of the extract for the HPLC analysis

Green tea leaves were extracted according to the following procedure: 1.0 g (accurate weight) of the crushed raw material was extracted two times by 60% ethanol in the ratio of 1:20 using the maceration method. The extracts obtained were filtrated and transferred into 50.0 mL measuring flask and made up to the mark with the same solvent.

Spectrophotometric measurements were carried out using a UV-1000 single beam spectrophotometer (China) with the pair of S90-309Q quartz square cells.

Preparation of the extract for the spectrophotometric analysis

To determine the total catechins content in green tea leaves, 5 g (accurate weight) of the crushed raw material was taken and placed in a 250 mL flask with ground glass joint, 100 mL of 70% ethanol was poured into the flask, and the mixture was boiled on a water bath for 1 hour. The extraction was repeated once more. After cooling the solution was quantitatively transferred into a 250 mL volumetric flask and diluted to the volume with 70% ethanol (*solution A*). 1 mL of the *solution A* was mixed with 7.5 mL of 1% vanillin solution in 96% ethanol in a 25 mL volumetric flask. Then the solution was diluted to the volume with 0.5 M HCl in 96% ethanol solution. The mixture was analyzed at 505 nm wavelength after standing for 30 min as the compensation liquid was 70% ethanol [20].

To plot the calibration curve of the dependence of absorbance on the amount of epigallocatechin-3-O-gallate, the stock solution (10 mg/mL) was prepared by dissolving 250.0 mg of (−)-epigallocatechin gallate in 96% ethanol, and the solution was diluted to 25.0 mL with the same solvent. The stock solution was diluted with the solvent to prepare the model solutions 1–5 with the concentrations of 100; 150; 200; 300; 400 μg/mL, respectively.

The total catechins content (X) in dry green tea leaves with reference to epigallocatechin-3-O-gallate was calculated in percent according to the following equation:

$$X\,(\%) = \frac{C_x \cdot 250.0 \cdot 25.0 \cdot 100 \cdot 100}{m_s \cdot 1.0 \cdot (100 - W)},$$

where, C_x – is the concentration of epigallocatechin-3-O-gallate according to the calibration curve, C × 10^{-6} (Fig. 3); m_s – is the sample weight, g; W – is the percentage of moisture in the raw material.

Results and discussion

Catechins were completely separated by the chromatographic conditions described above. The elution order of components is indicated as follows: EGC – 13.013 min, C – 13.780 min, EC – 16.494 min, EGCG – 17.686 min, ECG – 20.754 min (Table 1).

Fig. 1 shows the chromatogram of the green tea extract. The peaks of the substances were detected by a UV detector at a wavelength of 255 nm. EGC, C, EC, EGCG and ECG were identified on the chromatograms of the green tea extract by the indices of similarity with the standards used in the study.

The results obtained showed that EGCG is the catechin in the highest concentration, whereas content of C is the lowest. According to their amounts catechins can be arranged in the following row: EGCG > EGC > ECG > EC > C. The total content of catechins was 30.56% in green tea leaves by HPLC. The results are shown in Tables 2 and 3.

The content of catechins in the sample analyzed differed from that one described by *Ahmad et al.* [19]. They stated EGCG amount to be 12.1–17.7%, EC – 5.48–8.60%, EGC – 4.26–6.40% and ECG – 1.32–1.81% in green tea leaves. If we compare the results with our findings, it can be seen that the amount of EGCG and EC was higher, while the content of EGC and ECG

Table 1

Identification of substances in the extract of green tea leaves, which peaks are indicated in Fig. 1

Number of the peak in Fig. 1	Retention time, min	The similarity index of the retention time, I_T	The spectral similarity index, I_L	Identification
1	13.013	0.720	0.875	epigallocatechin
2	13.780	0.850	0.996	catechin
3	16.494	0.776	0.851	epicatechin
4	17.686	0.812	0.990	epigallocatechin gallate
5	20.754	0.732	0.814	epicatechin gallate

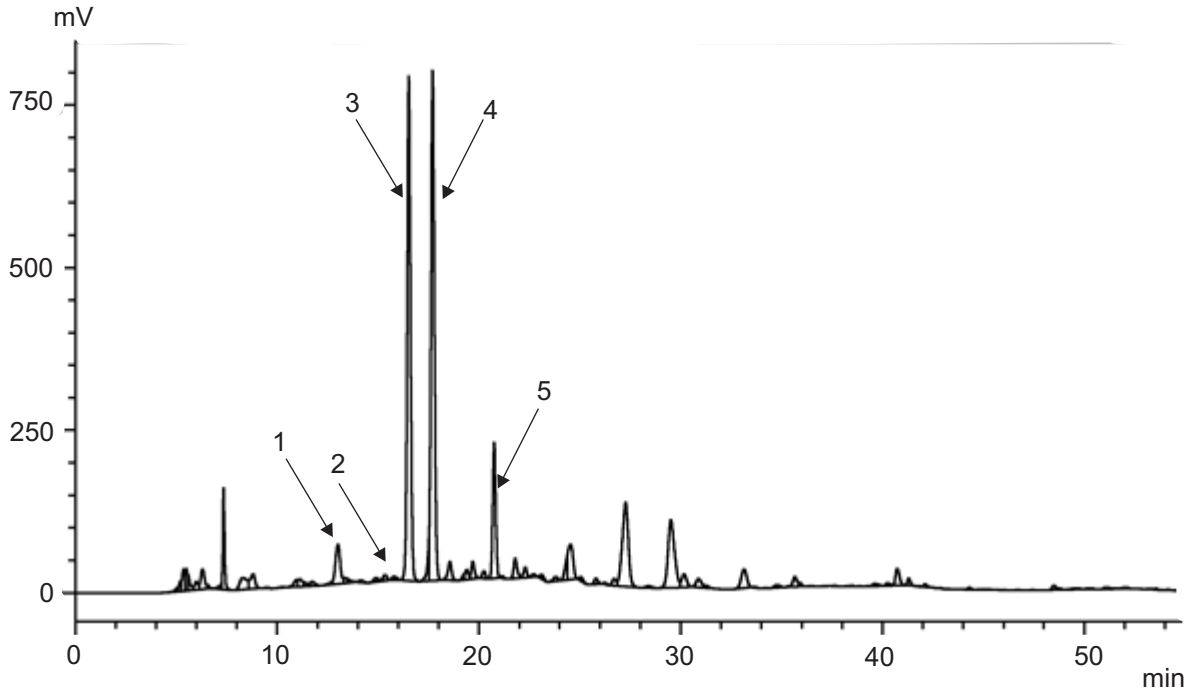

Fig. 1. The chromatogram of the extract of green tea leaves obtained at a wavelength of 255 nm

was lower in the study by *Ahmad et al*. The differences may be related with the sample preparation method since different brewing times, the ratios of leaves/extractant were used. The comparison of the results is sometimes difficult due to the lack of uniformity on the conditions used to prepare the extracts, as well as different geographical origin of the raw material. Moreover, leaves age is also a very important factor as old leaves contain much less EGCG and total catechins than young ones.

Catechins are able to react with the vanillin reagent to form red colored complexes due to the presence of phloroglucinol groups in the structure of catechins [20]. The reaction of vanillin with catechins is specific; flavonoids and carboxylic acids do not react with it. As shown in Fig. 2, the spectra for the vanillin complexes with either EGCG or the green tea leaves extract have nearly the same absorption maxima at about 505 nm.

The calibration curve was plotted using epigallocatechin gallate. Linearity was proven in the concentration range of (100–400) × 10^{-6} g/mL, the calibration equation was y = 0.0025x – 0.0851 (r = 0.9975) (Fig. 3). The total catechin content in green tea leaves found by spectrophotometry method was 24.79±1.12% (Table 3).

The statistical comparison was used for the results obtained by the two methods. According to these results

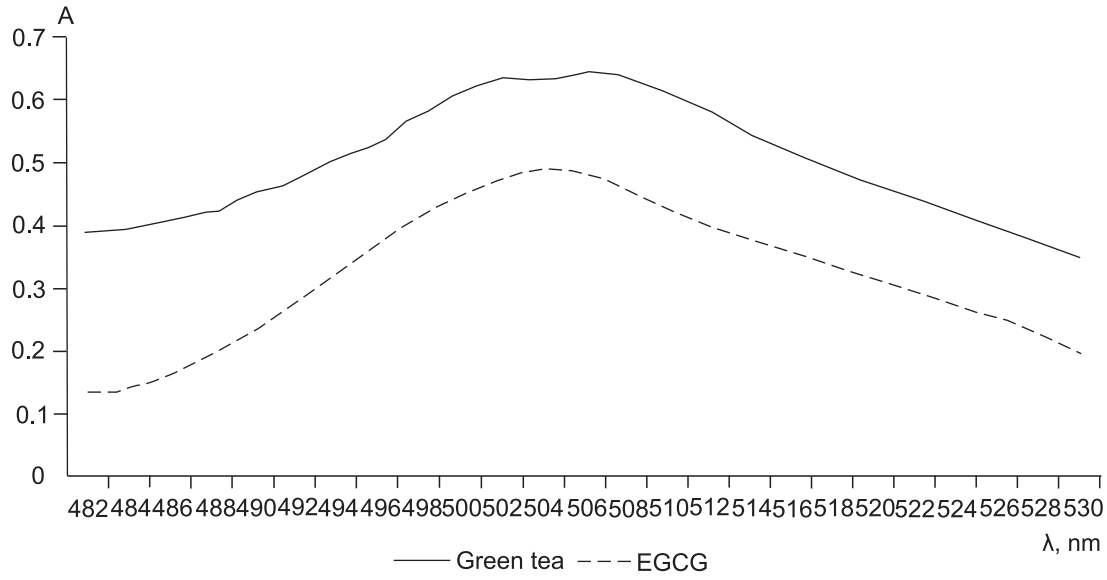

Fig. 2. Spectra of the vanillin complex with either the green tea leaves extract and EGCG

Table 2

The concentration of catechins in green tea leaves by HPLC

Catechins	The content of catechins in dry tea leaves, %
Epigallocatechin gallate	10.85 ± 0.11
Epicatechin	2.95 ± 0.04
Catechin	0.61 ± 0.01
Epigallocatechin	8.03 ± 0.10
Epicatechin gallate	8.12 ± 0.10
The total catechins	30.56 ± 0.59

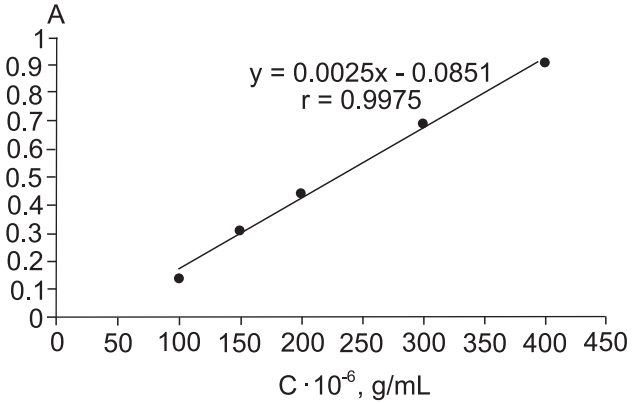

Fig. 3. The calibration curve of the absorbance against the concentration of epigallocatechin-3-O-gallate

Table 3

Metrological characteristics of the quantitative determination of the total catechin content in green tea leaves by spectrophotometry and HPLC

Method	$\bar{x}$	S^2	S	$S_{\bar{x}}$	Δx	ε,%	$\bar{x} + \Delta x$
Spectrophotometry	24.79	0.2950	0.5431	0.2429	1.07	2.73	24.79±1.07
HPLC	30.56	0.2255	0.4749	0.2124	0.59	1.94	30.56±0.59

Notes: $\bar{x}$ – mean, S^2 – dispersion, S – standard deviation, $S\bar{x}$ – standard deviation of the mean, Δx – confidential interval, ε – relative uncertainties of the result of a particular determination.

Table 4

The results of *t*- and *F*-tests when comparing HPLC and spectrophotometric methods

Method	$\bar{x}$, %	n	S^2	F_{tabl}	F_{calc}	t_{tabl}	t_{calc}
HPLC	30.56	5	0.75	6.39	0.46	2.31	1.09
Spectrophotometry	24.79		1.62				

Notes: F_{tabl} – value of theoretical Fisher criteria, F_{calc} – value of practical Fisher criteria, t_{tabl} – value of theoretical Student's criteria, t_{calc} – value of practical Student's criteria, n – number of samples, S^2 – dispersion of the samples

there was no significant difference between the spectrophotometric and HPLC methods (t_{calc} = 1.09, F_{calc} = 0.46) since the calculated *t* and *F* values did not exceed the theoretical ones (t_{tabl} = 2.31, F_{tabl} = 6.39). The results are shown in Table 4.

However, there was a difference between spectrophotometric and HPLC methods in the total content of catechins. The total catechins content in dry green tea leaves is higher by HPLC than that one obtained by the spectrophotometric method. This can be explained by their different extraction procedure, sample preparation, and an unstable complex between catechins and vanillin. Moreover, Table 3 demonstrates that HPLC is more accurate method than spectrophotometry.

Conclusions

The qualitative composition and the quantitative content of catechins have been determined in the extract from green tea leaves by HPLC and spectrophotometry methods. Both of the methods can be used to determine the total catechin content in green tea leaves. The high content of catechins makes the extract promising for further study and creation of new herbal medicinal products and dietary supplements. The results obtained will be used for standardization of green tea leaves and for future pharmacological research of its extract.

Conflict of interests: the authors have no conflict of interests to declare.

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