Study of flavonoids and phenolic acids in green tea leaves

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

The aim of work is study qualitative composition and quantitative content of flavonoids and phenolic acids in green tea leaves.

Material and methods. The object of the study was green tea leaves, which were collected in Anhui Province, China. The analysis of 60 % ethanolic extract from green tea leaves was performed by high-performance liquid chromatography using a Prominence LC-20 Shimadzu chromatographic system (Japan) with an SPD-20AV spectrophotometric detector, an Agilent Technologies Microsorb-MV-150 column (reversed-phase, C18 modified silica gel, length – 250 mm, diameter – 4.6 mm, particles size – 5 μm). Identification of substances in the extract was carried out by comparing the retention time and the spectral characteristics of the test substances with the same characteristics of the reference standards.

Results. 13 compounds were identified and determined by high-performance liquid chromatography. Among flavonoid aglycones quantitatively dominated by quercetin (0.35 %), in the case of flavonoid glycosides, it was luteolin-6-C-glycoside (1.30 %) and among phenolic acids, it was gallic acid (5.21 %).

Conclusions. The qualitative composition, quantitative content of flavonoids and phenolic acids in the green tea leaves were determined by high-performance liquid chromatography. According to HPLC, the content of flavonoids in green tea leaves was higher than the content of phenolic acids.

Key words: green tea, leaves, flavonoids, phenolic acids, high-performance liquid chromatography.

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Дослідження флавоноїдів і фенолокислот у зеленого чаю листі

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Мета роботи – визначити якісний склад і кількісний уміст флавоноїдів і фенолокислот у зеленого чаю листі.

Матеріали та методи. Об'єкт дослідження – зеленого чаю листя, що зібране в провінції Аньхой, КНР. Аналіз 60 % спиртової витяжки зеленого чаю листя здійснили методом високоефективної рідинної хроматографії за допомогою хроматографічної системи Prominence LC-20 Shimadzu (Японія) зі спектрофотометричним детектором SPD-20AV, колонка Agilent Technologies Microsorb-MV-150 (С18 модифікований силікагель, довжина – 250 мм, діаметр – 4,6 мм, розмір зерен сорбенту – 5 мкм). Ідентифікацію речовин у витяжці виконали шляхом порівняння часу утримування та спектральних характеристик речовин, що досліджували, зі стандартами.

Результати. У зеленого чаю листі ідентифікували 13 сполук, визначили їхній кількісний уміст. Серед агліконів флавоноїдів кількісно переважав кверцетин (0,35 %), а з-поміж глікозидів флавоноїдів – лютеолін-6-С-глюкозид (1,30 %). Із фенольних кислот основна сполука – галова кислота (5,21 %).

Висновки. Встановили якісний склад і кількісний уміст флавоноїдів, фенольних кислот у зеленого чаю листі методом високоефективної рідинної хроматографії. Вміст флавоноїдів у зеленого чаю листі перевищував такий для фенольних кислот.

Ключові слова: зелений чай, листя, флавоноїди, фенолокислоти, високоефективна рідинна хроматографія.

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Исследование флавоноидов и фенолокислот в зелёного чая листьях

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Цель работы – определение качественного состава и количественного содержания флавоноидов и фенолокислот в зелёного чая листьях.

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Key words: green tea, leaves, flavonoids, phenolic acids, high-performance liquid chromatography.

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Материалы и методы. Объект исследования – зелёного чая листья, собранные в провинции Аньхой, КНР. Анализ 60 % спиртовой вытяжки зелёного чая листьев провели методом высокоэффективной жидкостной хроматографии с помощью хроматографической системы Prominence LC-20 Shimadzu (Япония) со спектрофотометрическим детектором SPD-20AV, колонка Agilent Technologies Microsorb-MV-150 (C18 модифицированный силикагель, длина – 250 мм, диаметр – 4,6 мм, размер зёрен сорбента – 5 мкм). Вещества в вытяжке идентифицировали путём сравнения времени удерживания и спектральных характеристик исследуемых веществ со стандартами.

Результаты. В зелёного чая листьях идентифицировали 13 соединений, определили их количественное содержание методом высокоэффективной жидкостной хроматографии. Среди агликонов флавоноидов количественно преобладал кверцетин (0,35 %), а из гликозидов флавоноидов – лютеолин-6-С-глюкозид (1,30 %). Среди фенольных кислот доминирующее соединение – галловая кислота (5,21 %).

Выводы. Определили качественный состав и количественное содержание флавоноидов и фенольных кислот в зелёного чая листьях методом высокоэффективной жидкостной хроматографии. Содержание флавоноидов в зелёного чая листьях превышало содержание фенольных кислот.

Ключевые слова: зелёный чай, листья, флавоноиды, фенолокислоты, высокоэффективная жидкостная хроматография.

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Tea (*Camella sinensis L.*) is originated in China, dates back several thousand years. Tea composition varies with climate, season, tea variety, age of the leaf and horticultural practices [1]. A lot of epidemiological and preclinical studies have demonstrated that drinking tea may reduce the risk of cancer and cardiovascular disease [2,3].

Green tea leaves contain numerous bioactive compounds, among which catechins or flavan-3-ols are the most thoroughly investigated. Nevertheless, not only flavan-3-ols are contained in green tea leaves. Tea is rich in aglycones and glycosides of flavanols, flavanones, flavones, and phenolic acids [4,5]. Available scientific studies indicate that seven flavone glycosides were determined in leaves by high-performance liquid chromatography (HPLC), five apigenin compounds (apigenin-6,8-C-diglucoside, apigenin-6-C-glucoside-8-C-arabinoside, apigenin-6-C-arabinoside-8-C-glucoside, apigenin-8-C-glucoside, and apigenin-6-C-glucoside) as well as two luteolin compounds (luteolin-6-C-glucoside and luteolin-8-C-glucoside). In this study, the total amount of flavone glycosides was between 0.48 g/kg and 2.69 g/kg [6].

Literature shows that flavonols in tea are mainly present in the form of mono-, di-, and triglycosides as well as kaempferol, myricetin, and quercetin have been detected in several studies [7,8]. The total content of flavanols can be in the range of 1.0 % to 4.0 % in dry green tea leaves [9]. Moreover, several scientific research have reported about flavanones in the composition of green tea leaves [10,11]. Phenolic acids are represented by theogallin, gallic, ferulic, cinnamic acids, as well as the total amount of phenolic acid and its derivatives, which is in the range from 0.1–2.0 % in dry material [12].

Aim

The aim of the study is determined qualitative composition and quantitative content of flavonoids and phenolic acids in green tea leaves.

Materials and methods

The object of the study was green tea leaves, which were collected in Anhui Province, China.

Caffeic, gallic, ferulic, cinnamic acids, myricetin-3-O-glycoside, quercetin, quercetin-3-O-rutenoside, naringenin, naringin, hesperidin, hesperetin, luteolin-6-C-glycoside, apigenin-8-C-glycoside were purchased from Sigma-Aldrich. The methanol was HPLC grade and other chemicals were analytical grade.

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

The analysis of plant samples is quite complicated as above all, the plant matrices are often very complex, and identify each substance is impossible, in addition, some standards of substances are very costly. That is why the method of similarity indices was used to provide the analysis. The similarity indices are calculated according to the following formulas [13]:

$$I_T = 1 - |T_{st} - T_u|$$
$$I_{255} = 1 - |h_{255st} - h_{255u}|$$
$$I_{286} = 1 - |h_{286st} - h_{286u}|$$
$$I_{350} = 1 - |h_{350st} - h_{350u}|$$

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

The spectral characteristic of substance is the ratio of the peak height of chosen wavelength 255 nm, 268 nm, and 350 nm to the peak height of test substance at 225 nm [14]. For identification analyzed substance the index similarly is chosen among three, which has the lowest value. In order to

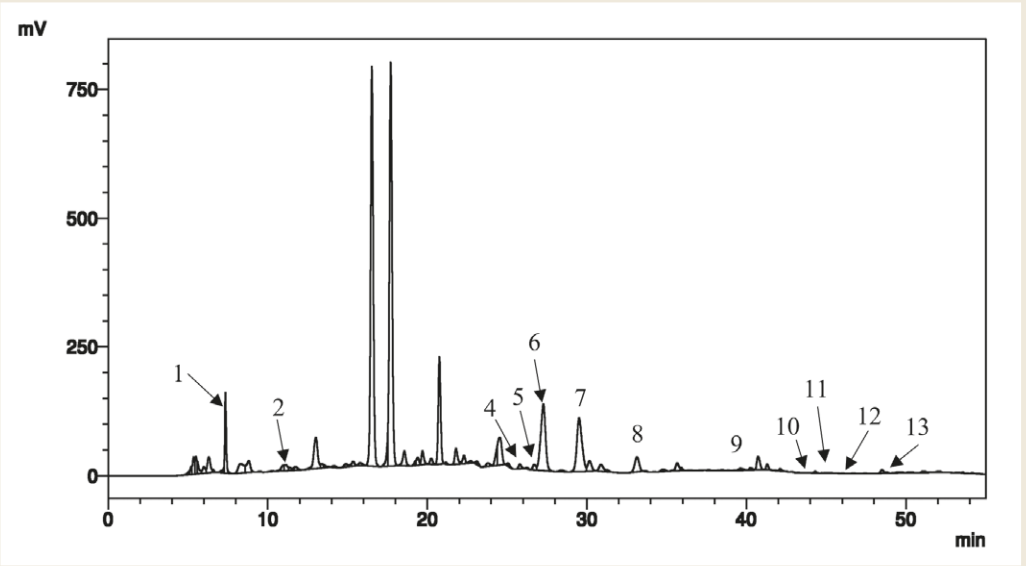

Fig. 1. Chromatogram of test solution of green tea leaves extract, obtained at a wavelength of detection of 255 nm.

identify the chromatographic peaks, the value of the similarity index must be greater than 0.7, if it is not so this peak is unidentified. Some peaks can be outside of the flavonoid range it indicates that this substance is not a flavonoid structure and it can be sorted out to unidentified [15].

Extraction of green tea leaves was made according to the following procedure: 1.0 g of crushed raw material was extracted by 60 % ethanol in a ratio of 1:20 using method maceration. The resulting extract was filtered through a filter. The raw material was extracted twice with new portions of the solvent, after that the extracts were combined.

Statistical analysis was performed in Microsoft Excel 2010 with the accepted significance level $\alpha = 0.05$. Results were expressed as mean ± confident interval from five measurements.

Results

The figure shows a chromatogram of green tea extract. Detection of the substance's peaks was provided by a UV detector at a wavelength of 255 nm. 120 peaks were identified, which were analyzed by indices of similarity to the standards, 21 peaks were included in the group of unidentified. The chromatogram shows the main peaks, the numbers of which coincide with the numbers of those identified compounds in *Table 1*. According to the described previously procedure that is based on high similarity indices with standard substances I_T and I_L, 6 flavonoid glycosides were identified quercetin-3-O-rutenoside, kaempherol-7-O-glycoside, myricetin-3-O-glycoside, hesperidin, luteolin-6-C-glycoside, apigenin-8-C-glycoside as well as 3 flavonoid aglycones – quercetin, naringenin, hesperitin (*Table 1*).

In our research, the number of flavonoid glycosides was: luteolin-6-C-glycoside (1.30 %), hesperidin (1.19 %), apigenin-8-C-glycoside (0.98 %), myricetin-3-O-glycoside (0.86 %), quercetin-3-O-rutenoside (0.62 %), kaempherol-7-O-glycoside (0.51 %), as well as the total content of flavonoid glycosides in green tea leaves was found 5.46 % (*Table 2*). The total amount of flavonoid aglycones and glycosides was 0.41 % and 5.46 %, respectively. The phenolic acids were represented by gallic acid (5.21 %), caffeic acid (0.16 %), cinnamic acid (0.02 %), and ferulic acid (0.01 %). The total content of phenolic acids was 5.39 % in dry raw material.

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

Number of peak on *Fig. 1*	Retention time, min	Similarity index, I_L	Identification
1	8.815	0.826	gallic acid
2	14.850	0.921	hesperidin
3	22.295	0.816	apigenin-8-C-glycoside
4	24.372	0.859	myricetin-3-O-glycoside
5	25.801	0.863	caffeic acid
6	27.275	0.988	kaempherol-7-O-glycoside
7	29.915	0.943	quercetin-3-O-rutenoside
8	33.147	0.888	luteolin-6-C-glycoside
9	40.327	0.527	cinnamic acid
10	40.727	0.760	quercetin
11	40.977	0.725	naringenin
12	41.788	0.928	hesperetin
13	45.681	0.793	ferulic acid

Discussion

In scientific research [16] it was estimated that the amount of myricetin-3-O-glycoside was 0.083–0.159 %, quercetin-3-O-rutenoside was 0.15–0.48 %, kaempherol-7-O-glycoside was 0.16–0.33 % and quercetin was 0.10–0.50 % in dry material. In this study the content of quercetin-3-O-rutenoside was the greatest. The available research [17] shows

Table 2. The content of flavonoid glycosides in extract green tea leaves by HPLC-UV

Glycoside	Content, % in dry raw material
kaempherol-7-O-glycoside	0.17 ± 0.01
quercetin-3-O-rutenoside	9.35 ± 0.19
myricetin-3-O-glycoside	2.15 ± 0.04
apigenin-8-C-glycoside	2.45 ± 0.05
hesperidin	1.98 ± 0.04
luteolin-6-C-glycoside	3.25 ± 0.06
Total flavonoid glycoside	19.35

Table 3. The content of flavonoid aglycones in extract green tea leaves by HPLC-UV

Aglycone	Content, % in dry raw material
quercetin	0.35 ± 0.005
naringenin	0.02 ± 0.001
hesperitin	0.04 ± 0.001
Total flavonoid aglycone	0.41

Table 4. The content of phenolic acids in extract green tea leaves by HPLC-UV

Phenolic acid	Content, % in dry raw material
Gallic acid	5.210 ± 0.052
Caffeic acid	0.160 ± 0.002
Cinnamic acid	0.020 ± 0.001
Ferulic acid	0.010 ± 0.001
Total phenolic acids	5.39

that the content of naringenin was 0.01–0.11 %, hesperitin was 0.01–0.07 %, hesperidin was 0.31–0.81 %. The previous investigation [18] represents the gallic acid was in the range from 2.0 % to 6.0 %.

According to results, the luteolin-6-C-glycoside had the highest concentration among other glycosides. The major constituent among aglycones was quercetin, whereas other aglycones were present at the lowest level (*Table 3*). Obtained results indicated that flavonoid glycosides were predominated in green tea leaves.

The main compound of phenolic acids was gallic acid, the high concentration among others can be explained by the releasing gallic acid from gallaylated catechin-derived species (*Table 4*). Compared results represent that analyzed green tea leaves are accumulated more flavonoids than phenolic acids.

The differences in results of research can be related with sample preparation method, since different brewing times, ratio leaves/extractant were used, species of tea, climate and geographical position. This work is a contribution for the chemical composition of green tea leaves. Moreover, obtained data can be used further for standardization green tea leaves.

Conclusions

1. The qualitative composition, quantitative content of flavonoids and phenolic acids green tea leaves were determined by high-performance liquid chromatography.

2. According to HPLC, the content of flavonoids in green tea leaves was higher than the content of phenolic acids.

Prospects for further research. The obtained data on the composition of phenolic acids and flavonoids of green tea leaves will be used for further standardization of the obtained extract and indicate the possibility of creating phytopreparations and food additives on the extract.

Conflicts of interest: authors have no conflict of interest to declare.
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