UDC 615.32:582.687.21:615.451.1:54.061/.062

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DOI: 10.33617/2522-9680-2022-1-48

To cite this article: Maslov O., Kolysnik S., Komisarenko M., Golik M., Antonenko O. (2022). Vyvchennia khimichnoho skladu i antyoksydantnoi aktyvnosti nastoiky, nastiiu z lystia zelenoho chaiu [Study of chemical composition and antioxidant activity of tincture, infusion of green tea leaves]. *Fitoterapiia. Chasopys – Phytotherapy. Journal*, 1, 48–53, doi: 10.33617/2522-9680-2022-1-48

STUDY OF CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF TINCTURE, INFUSION OF GREEN TEA LEAVES

Aim. *To provide the qualitative, quantitative analysis of phenolic compounds of tincture and infusion of green tea leaves and determine its antioxidant activity.*

Material and methods. *Green tea leaves of species Chun Myn were the object of the study, which were collected in Anhui province, China from March to April. The qualitative analysis was performed by thin layer chromatography (TLC), spectrophotometry was used for the quantitative determination on the spectrophotometer UV-1000 (China), antioxidant activity was found by potentiometric method on pH meter HANNA 2550 (Germany) with a combined platinum electrode EZDO 50 PO (Taiwan). Tincture of green tea leaves was obtained by the maceration method with 60% ethanol in ratio raw material/solvent 1:10 (mass of dry leaves 10,0 g), infusion was obtained by the same way only with distilled water according to State Pharmacopeia of Ukraine.*

Results and discussion. *As result of study it was found that the total content of phenolic compounds was 7.90, 3.54 mg/mL, catechins – 8.43, 3.36 mg/mL, flavonoids – 0.25, 0.38 mg/mL, hydroxycinnamic acids – 0.45, 0.36 mg/mL, which was determined with spectrophotometrical method and antioxidant activity – 48.27, 18.23 mmol-eqv./$m_{dry\ res.}$ that was measured with potentiometrical method in tincture, infusion of green tea leaves, respectively.*

Conclusions. *The qualitative and quantitative analysis of phenolic compounds, catechins, flavonoids and hydroxycinnamic acids of tincture and infusion green tea leaves has been provided. The research has revealed that tincture has contained phenolic compounds in 55%, catechins in 60%, hydroxycinnamic acids in 13% more and flavonoids in 34% less than in infusion. The antioxidant activity of green tea tincture in 38% higher than in infusion. The 60% ethanol is more appropriate solvent for extraction catechins than water, which can be used for developing and obtaining medicines, dietary supplements and cosmetologically products.*

Key words: *green tea leaves, analysis, infusion, tincture, antioxidant activity.*

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Бібліографічний опис статті: Маслов О., Колісник С., Комісаренко М., Голік М., Антоненко О. (2022). Вивчення хімічного складу і антиоксидантної активності настойки, настію з листя зеленого чаю. *Фітотерапія. Часопис*, 1, 48–53, doi: 10.33617/2522-9680-2022-1-48

ВИВЧЕННЯ ХІМІЧНОГО СКЛАДУ І АНТИОКСИДАНТНОЇ АКТИВНОСТІ НАСТОЙКИ, НАСТІЮ З ЛИСТЯ ЗЕЛЕНОГО ЧАЮ

Мета. *Провести якісний, кількісний аналіз фенольних сполук настойки та настою отриманого з листя зеленого чаю.*

Матеріали і методи*. Об'єктом дослідження було листя зеленого чаю сорту Чун Мін, які були зібрані в провінції Анхуй, Китай з березня по квітень. Якісний аналіз проводили методом тонкошарової хроматографії (ТСХ), кількісне визначення проводили спектрофотометричним методом на спектрофотометрі UV-1000 (Китаї), антиоксидантну активність визначили потенціометричним методом на pH метрі HANNA 2550 (Німеччина) з комбінованим платиновим електродом EZDO 50 PO (Тайвань). Настоянку листя зеленого чаю отримували методом мацерації з 60% етиловим спиртом у співвідношенні сировина/розчинник 1:10 (маса сухого листя 10,0 г), настій отримували таким же способом тільки з дистильованою водою згідно до Державної Фармакопеї України.*

Результати та їх обговорення. *В результаті дослідження встановлено, що сумарний вміст фенольних сполук становив 8,43, 3,36 мг/мл, флавоноїдів – 0,25, 0,38 мг/мл, гідроксикоричних кислот – 0,45, 0,36 мг/мл визначенні спектрофотометричним методом і антиоксидантна активність – 48,27, 18,23 ммоль-екв./$m_{сух. зал.}$ була виміряна потенціометричним методом у настойці, настію із листя зеленого чаю, відповідно.*

Висновки*. Проведено якісний та кількісний аналіз фенольних сполук, катехінів, флавоноїдів і гідроксикоричних кислот у настоянці і настою листя зеленого чаю. Встановлено, що вміст фенольних сполук, катехінів та гідроксикоричних кислот більше в настоянці на 55, 60 та 13%, а флавоноїдів у 34% менше ніж у настій. Антиоксидантна активність настойки зеленого чаю на 38% вища, ніж у настою. 60% етиловий спирт є більш відповідним розчинником для екстракції катехінів, ніж вода, що може бути використане для розробки та одержання лікарських засобів, дієтичних добавок та косметологічних продуктів.*

Ключові слова: *листя зеленого чаю, аналіз, настій, настойка, антиоксидантна активність.*

Introduction. Green tea leaves (*Camellia sinensis L.*) are included in pharmacopeias of USA and Europe (Maslov, 2021, pp. 25-34, The United States Pharmacopea 38). Tea is one of the most popular beverages over the world. Green tea is originated in China, dates back several thousand years. A variety of epidemiological researches have represented that drinking tea reduce the risk of cancer and cardiovascular diseases (Kochman, 2020, p. 85). Today on Ukraine pharmaceutical market we can observe 47 dietary supplements with green tea leaves extract where leading position take USA, mainly they are applied as antioxidant and weight loss products.

In traditional medicine of China green tea tincture and infusion are prescribed for treating hypertension, atherosclerosis, diabetes mellitus, arthritis (Cooper, 2005, pp. 521-528). Moreover, green tea tincture is used to protect against osteoporosis in older women due to increasing bone mineral density (Huang, 2020, pp. 1136). In addition, it has proved that green tea infusion has antiviral effect that may support the prevention and regulate immune response infection diseases, including COVID-19 (Senanayake, 2013, pp. 1529-1541).

Green tea leaves contain variety of polyphenols such as catechins (30 – 35%), flavanols (1 – 2.5), flavanones (1.5 – 3%), phenolic acids (2 – 5%), except phenolic compounds there are caffeine (1.5 – 2.5%), amino acids (1 – 5.5%), organic acids (1 – 1.8%) (Maslov, 2021, pp. 25-34, Maslov, 2021, pp. 287-291). The catechins are dominated among all phenolic compounds.

The aim of the study was provided the qualitative, quantitative analysis of phenolic compounds of tincture and infusion of green tea leaves and determine its antioxidant activity.

Material and methods. Green tea leaves of spices Chun Myn were the object of the study, which were collected in Anhui province, China from March to May.

Standards of epicatechin, epigallocatechin-3-O-gallete, epicatechin-3-O-gallate, epigallocatechin, rutin, caffeic acid, chlorogenic acid were obtained from Sigma-Aldrich. All solvent and other chemical were of analytical grade. «Sorbfil –PTSH–AF–A–UV» (Russia) plates were used for TLC analysis.

The pH meter HANNA 2550 (Germany) with a combined platinum electrode EZDO 50 PO (Taiwan) were applied to conduct potentiometric measurements. Quantitative analysis of biological active compounds was provided on UV-spectrophotometer UV – 1000 (China) with matched 1 cm quarts cell.

Weighing was carried out using digital analytical balance AN100 (AXIS, Poland) with d = 0.0001 g.

Tincture of green tea leaves was obtained by the maceration method with 60% ethanol in ratio raw material/solvent 1:10 (mass of dry leaves 10,0 g), infusion was obtained by the same way only with distilled water according to State Pharmacopeia of Ukraine (SPhU).

The amount of dry residue of tincture and infusion were determined by gravimetric method according to SPhU.

To identify catechinds, the developing system consisting of toluene/methanol/formic acid (9 : 9 : 2) was used; for flavonoids and hydroxycinnamic acids the developing system was ethyl acetate/ glacial acetic acid/formic acid/water (100 : 11 : 11 : 26). The samples were spotted using a 10 μL micro-pipette with 30 μL of the test solutions of the tincture and infusion, 10 μL of the standards solutions. The sample plates were air dried, then placed in chromatographic chambers, which were presaturated with the developing systems and chromatographed in ascending order. When the front of the solvent passed about 8 cm, the plates were removed from the chambers and dried in air for 30 min. Catechins were detected in UV light at wavelengths of 254 and 325 nm. For the final determination of catechins, the dried plates were treated with 1% vanillin solution of 1M hydrochloric acid, flavonoids and hydroxycinnamic acids were detected by 10% solution of KOH in 50% ethanol.

The total phenols were measured by the Folin-Ciocaltau assay at 760 nm (Al-Shwaiyat, 2018, pp. 135 – 142). The total phenols in tincture and infusion, expressed as gallic acid was calculated according to the following equation:

$$X\,(mg\ /\ mL) = \frac{C_x \cdot K_{dil} \cdot 1000}{V}$$

where, C_x – concentration of gallic acid according to calibration curve, $C \cdot 10^{-6}$, g/mL; V – volume of tincture and infusion, mL; K_{dil} – coefficient of dilution.

The vanillin reagent assay was applied to find out the total catechins (Maslov, 2021, pp. 232 – 233), the absorbance was measured at 505 nm. The total catechins in tincture and infusion, expressed as epigallocatechin-3-O-gallate was calculated according to following equation:

$$X\,(mg\ /\ mL) = \frac{C_x \cdot K_{dil} \cdot 1000}{V}$$

where, C_x – concentration of epigallocatechin-3-O-gallate according to calibration curve, $C \cdot 10^{-6}$ g/mL; V_{ext} – volume of tincture and infusion, mL; K_{dil} – coefficient of dilution.

The total flavonoids were determined using assay of complex formation with $AlCl_3$ at 417 nm (Maslov, 2021, pp. 215 – 219). The total flavonoids in tincture and infusion, expressed as rutin was calculated according to following equation:

$$X\,(mg\ /\ mL) = \frac{A \cdot K_{dil} \cdot 1000}{A_{st} \cdot \mathrm{V}}$$

where, A – absorbance of analyzed solution, A_{st} – absorbance of standard solution of rutin; V_{ext} – volume of tincture and infusion, mL; K_{dil} – coefficient of dilution.

The total hydroxycinnamic acid content was measured by assay of complex formation with $NaNO_2$-Na_2MoO_4 at 525 nm (Lukashenya, 2020, pp. 250 – 254). The total hydroxycinnamic acids in tincture and infusion, expressed as chlorogenic acid was calculated according to following equation:

$$X\,(mg\ /\ mL) = \frac{A \cdot K_{dil} \cdot 1000}{188 \cdot \mathrm{V}}$$

where, A – absorbance of analyzed solution, 188 – specific adsorption coefficient of chlorogenic acid; V – volume of tincture and infusion, mL; K_{dil} – coefficient of dilution.

Antioxidant activity of tincture and infusion was evaluated by potentiometric method (Maslov, 2021, pp. 35-42). Antioxidant activity was calculated according to the following equation and expressed as mmol-eqv./$m_{res\ dry\ weight}$:

$$\mathrm{AOA} = \frac{C_{ox} - \alpha \cdot C_{red}}{1 + \alpha} \cdot K_{dil} \cdot 10^3 \cdot \frac{m_1}{m_2}$$

where, $\alpha = C_{ox}/C_{red} \cdot 10^{(\Delta E - E\mathrm{ethanol})nF/2.3RT}$; C_{ox} – concentration of $K_3[Fe(CN)_6]$, mol/L; C_{red} – concentration of $K_4[Fe(CN)_6]$, mol/L; $E_{ethanol}$ – $0.0546 \cdot C_{\%}$ – 0.0091; $C_{\%}$ – concentration of ethanol; ΔE – change of potential; F = 96485.33 C/mol – Faraday constant; n = 1 – number of electrons in electrode reaction; R = 8.314 J/mol · K – universal gas constant; T – 298 K; K_{dil} – coefficient of dilution; m_1 – mass of dry weight residue; m_2 – mass of dry residue in 1.0 mL of tincture or infusion.

For all the experiments, five samples were analysed and all the assays were carried out in 5 times. The results were expressed as mean values with confident interval. The MS EXCEL 7.0 and STATISTIKA 6.0 were used to provide statistical analysis.

Results and discussion. The TLC method was used for identification catechins and other phenolic compounds. Catechins were detected in the toluene/methanol/formic acid (9 : 9 : 2) developing system. Substances were detected at wavelengths of 254 and 325 nm. The chromatogram showed the dominant bands with the value of R_f = 0.45 (epigallocatechin-3-O-gallate), R_f = 0.50 (epicatechin-3-O-gallate), R_f = 0.57 (epigallocatechin), R_f = 0.61 (epicatechin). The chromatogram was then sprayed by 1% vanillin solution of 1M hydrochloric acid; red bands of catechins with the same R_f values were also detected. For identification of flavonoids and hydroxycinnamic acids in the tincture and infusion the ethyl acetate/glacial acetic acid/formic acid/water (100 : 11 : 11 : 26) developing system was used. The TLC plate was sprayed with 10 % solution of KOH; as the result, yellow bands with values of Rf = 0.35 (rutin), blue fluorescence R_f = 0.40 (chlorogenic acid) and R_f = 0.45 (caffeic acid) appeared.

As shown in table 1, the highest content of phenolic compounds has found in tincture (7.90±0.16 mg/mL) than in infusion (3.54±0.10 mg/mL). Rodrigue M. A. et all (Rodrigue, 2016, 322 – 329) has established that total phenolic content was in the range from 1.10 – 1.15 mg/mL in green tea infusions. Comparing results with our study the amount of phenolic compounds in tincture and infusion is higher than in the above represented study.

Table 1 represents that content of catechins in tincture is greater in 60% than in infusion. In scientific research of Reto M. et. all (Reto, 2007, pp. 139 – 144), total amount of catechins was ranged from 0.40 to 1.13 mg/mL in infusions of different green tea. Compared to our study the total content of catechins is higher in 87% and infusion in 56% than in study of Reto M. et. all.

It has established that tincture has lower content of flavonoids (0.25±0.01 mg/mL) than in infusion (0.38±0.01 mg/mL) which can be explained by the high content of glycosides of flavonoids than its aglycones form. Rodrigue et. all (Rodrigue, 2016, 322 – 329) has found that total flavonoid content is 0.9 mg/mL in infusion of green tea. Comparing obtained results and Rodrigue et. all the total flavonoid content is lower in tincture and infusion of our study.

The highest content of hydroxycinnamic acids has observed in tincture (0.45±0.02 mg/mL) than in infusion (0.36±0.01 mg/mL).

According to obtain results phenolic compounds and catechins are dominated followed by hydroxycinnamic acids and flavonoids both in tincture and infusion.

In our recent study (Maslov, 2021, pp. 44 – 47) we have found that ethanol contributes to antioxidant activity regarding to that it has proposed the way of taking into

Table 1

The total content of phenolic, catechin, flavonoid and hydroxycinnamic acid compounds in green tea tincture and infusion

Sample	Dry residue, g	Total phenols, (mg/mL)	Total catechins, (mg/mL)	Total flavonoids, (mg/mL)	Total hydroxycinnamic acids, (mg/mL)
Tincture	0.9850	7.90±0.16	8.43±0.17	0.25±0.01	0.45±0.02
Infusion	0.4310	3.54±0.10	3.36±0.10	0.38±0.01	0.39±0.01

Table 2

Results of antioxidant activity of green tea leaves tincture and infusion

Sample	Antioxidant activity, mmol-eqv./$m_{res.\ dry\ weight}$	Conditional terms of antioxidant activity
Tincture	48.27	middle level
Infusion	18.23	below middle level

account the influence of ethanol. Table 2 represents that antioxidant activity of tincture is greater in 38% than infusion. We have elaborated the conditional terms of antioxidant activity (Maslov, 2021, pp. 215 – 219) which can help to classify antioxidants and develop the most appropriate technology of obtaining medicines, dietary supplements and cosmetologically products. According to this classification tincture has middle level of antioxidant activity while infusion – below middle level of antioxidant activity

Conclusions

1. The qualitative and quantitative analysis of phenolic compounds, catechins, flavonoids and hydroxycinnamic acids of tincture and infusion green tea has been provided.

2. The research has revealed that tincture has contained phenolic compounds in 55%, catechins in 60%, hydroxycinnamic acids in 13% more and flavonoids in 34% less than in infusion.

3. The antioxidant activity of green tea tincture in 38% higher than in infusion.

4. The 60% ethanol is more appropriate solvent for extraction catechins than water, which can be used for developing and obtaining medicines, dietary supplements and cosmetologically products.

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Стаття надійшла до редакції 08.12.2021.
Стаття прийнята до друку 24.01.2022.

Автори заявляють про відсутність конфлікту інтересів.
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УДК 615.322

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DOI: 10.33617/2522-9680-2022-1-53