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Catechins and antioxidant activity of tea products

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ABSTRACT

The paper presents the results of studies of catechins and antioxidant activities of extracts of various experimental and commercial types of tea by using High-performance liquid chromatography method (HPLC). The best solvent eluents of the stationary phase of this method and the two different detection waves have been established. The study presents catechins and antioxidant activities of 12 different kinds and origins of experimental and commercial tea extracts. The quantitative composition of the four catechins in commercial tea samples has been studied in detail. Due to the chemical composition of raw materials and processing technology, high values of antioxidant activity and amount of catechins - epigallocatechingallate and epigallocatechine in experimental and commercial samples of Georgian green, red and black tea have been determined.

Keywords: Tea, Catechins, Antioxidant activity, HPLC, Individual catechins, Tea leaf.

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Background

Tea is produced from the plant *Camelia sinensis* (L) O. Kuntze, as result of the technological processing, the most important taste properties and biological activity of which are determined by polyphenols, primarily catechins. Fundamental studies have confirmed the high P-vitamins activity, antioxidant, anti-inflammatory, antimicrobial, antiviral, anticancer and anticoagulant activities of these substances. The ability of catechins shows to alleviate cardiovascular, atherosclerotic, and hypertensive diseases, to reduce harmful level of cholesterol in the body. To a large extent, the positive action and quantity of epigallocatechingallate determines the health benefits of green tea. Tea consumption is correlates with low incidence of cardiovascular disease and cancer [1 - 9]. Green tea epigallocatechin gallate is characterized by a pronounced ability to stop obesity and metabolic syndrome [10 - 15]. High therapeutic

and prophylactic properties are also maintained by catechin conversion products - theaflavins [16]. Tea consumption can play an important role in providing the human body with antioxidants [17].

The antioxidant activity of tea is due to phenolic compounds and individual catechins, the amount and ratio of which depends on the plant variety, environmental conditions, the period of raw material production, the technological process and some other factors [18]. Tea quality control, in accordance with the requirements of international and national standards, provides for the determination of both the total number of polyphenols as well as catechins [19, 20].

Comparative data on the individual catechins and antioxidant activities of Georgian tea products by various technologies, including imported tea products, are quite scarce [21].

The aim of this paper is to study the individual

catechins and antioxidant activities of ready-made tea extracts of different types and origins.

Research objects and method

The following experimental and commercial tea products are used as research objects:

1. Tea leaf polyphenols concentrate;
2. Fixed tea leaf;
3. Experimental Georgian green tea;
4. “Greenfield” (Georgian green tea);
5. “Shemokmedi” (Georgian green tea);
6. “Royal Richards” (Chinese green tea);
6. “Gurieli” (Georgian green tea);
7. Experimental Georgian red tea;
8. Indian black tea (BOPF);
9. Experimental Georgian black tea;
10. Georgian black tea “Kolkhida”;
11. Black tea granulated (CTC).

Extracts are obtained in the same way as tea tasting and consumer tincture preparation, with single extraction of samples. Consequently, the obtained data do not claim a complete quantitative analysis of the compounds present in the plant material. To prepare the extracts for the analysis of antioxidant activities, 35 ml of boiling water is added to a 0.5 g tea sample. The extraction process takes 20 minutes. The 60 μ l sample is taken from the extracts obtained and antioxidant activities are determined. To obtain catechin analysis extracts, 0.5 ml of boiling water is added to a 0.5 g sample of tea, the duration of the process being 20 min. The 10 μ l samples taken from the extracts are analyzed by high

performance liquid chromatography. High performance liquid chromatograph Agilent 1260 Infinity (USA) and chromatographic column Supelco - C18 (25 cm $\times$ 4.6 mm, 5 μ m), temperature 250 C. Rolling phase - acetonitrile, 1% acetic acid dissolved in desalinized water to purify. Accepted using system Millipore (Merck, Germany) were used. Detection is performed using a single-signal ultraviolet detector at different wavelengths - 210 and 278 nm. The calibration curve is constructed at different concentrations of epigallocatechin gallate. The total amount of phenolic compounds and antioxidant activity in experimental and commercial tea products were determined using the relevant methods in the literature [19, 22, 23].

Statistical analysis

The results are expressed as mean $\pm$ SEM. Student's t-test was used to analyze level of statistical significance between groups. $P < 0.05$ was considered statistically significant.

Results and Discussion

The study results show a direct dependence between the antioxidant activity of tea extracts and the total amount of polyphenols (Fig. 1). Indicators distinguished the liquid concentrate of fixed tea leaf polyphenols made by special technological process [22]. According to a study of the total amount of antioxidant activities and polyphenols, tea samples

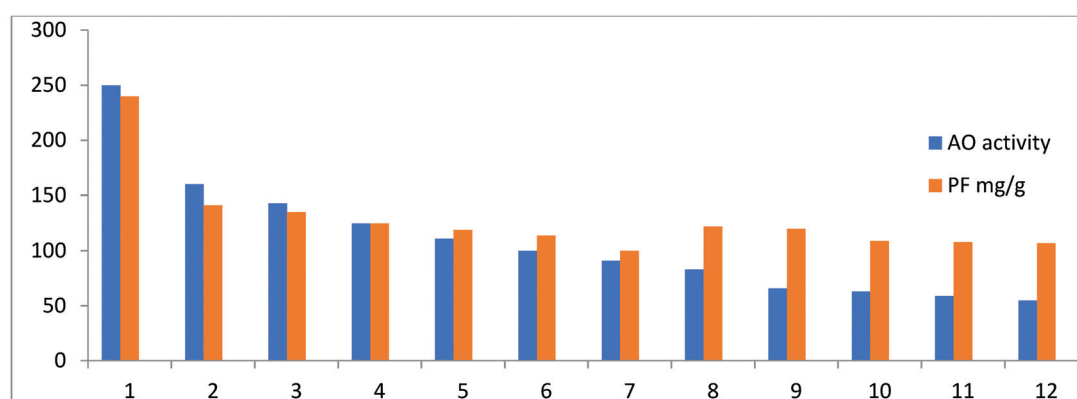


Fig. 1. Total amount of polyphenols (PF), mg/g and antioxidant activity (AO) $R=1/S \text{ sec.} \times 1000$ in tea

1-Tea leaf polyphenols concentrate; 2 - Fixed tea leaf; 3 - Experimental Georgian green tea; 4 - “Greenfield” (Georgian green tea); 5 - “Shemokmedi” (Georgian green tea); 6 - “Royal Richards” (Chinese green tea); 7 - “Gurieli” (Georgian green tea); 8 - Experimental Georgian red tea; 9 - Indian black tea (BOPF); 10 - Experimental Georgian black tea; 11 - Georgian black tea “Kolkhida”, 12 - Black tea granulated (CTC)

data were different. The total amount of polyphenols in black tea extracts was quite high as green tea, although they are characterized by fewer antioxidant activity properties. This is due to the fact that catechins of high antioxidant activity are oxidized in black tea extracts.

The total amount of polyphenols in black tea

sample extracts is also quite high, although this type of product is characterized by less antioxidant activity. This may be due to the lack of epigallocatechin gallate, which causes antioxidant activity in the extracts of these products, or the lack of secondary compounds that are also characterized by high antioxidant activity in vitro experiments.

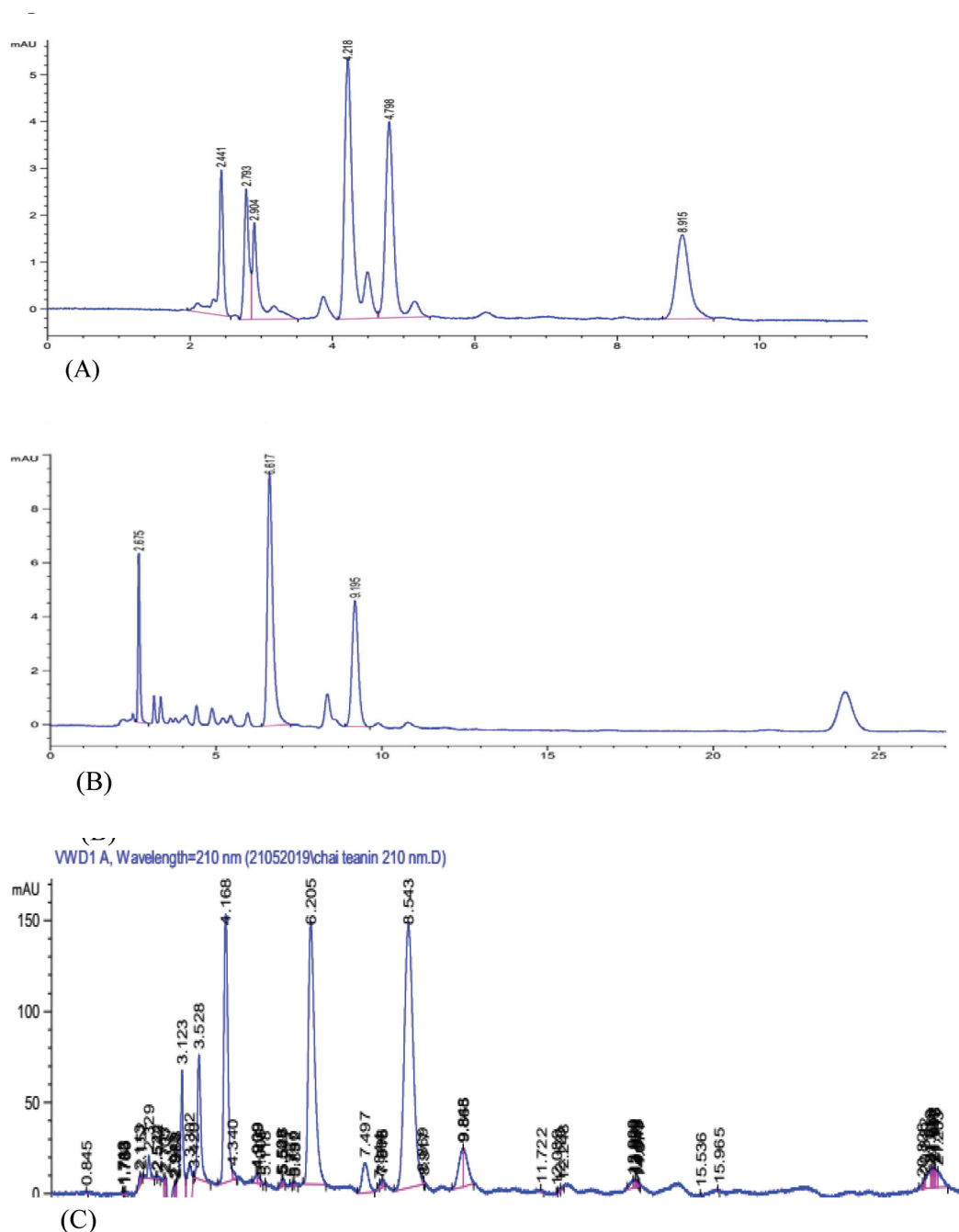


Fig. 2. Chromatographic profiles of green tea extracts according to different elements and detection wave:

A-Acetonitrile – water 1% acetic acid 15 : 85, detection at 278 nm;

B –Acetonitrile – water 1% acetic acid 20 : 80, detection at 278 nm;

C –Acetonitrile – water 1% acetic acid 15 : 85, detection at 210 nm

Fig. 2 presents the results of a study of the chromatographic separation of catechins under two different variants of the eluent ratio. At relatively high concentrations of acetonitrile (20:80) the division proceeds more rapidly, and the peak of the last catechin epicatechin gallate leaves the column earlier according to the retention of time. Although the eluent is acetonitrile - water, the chromatography time is longer at 15: 85, but the separation peaks are more pronounced. Especially in the beginning,

when the division is underway into major catechins. Important fact is that the peak of the epigallocatechin gallate is pronounced and can be counted most accurately. From the obtained data it can be seen that 210 Under the conditions of Nm wave detection, the intensity of the corresponding peaks of the catechins increases, while that of caffeine and other compounds decreases (Fig. 2 (C)). This is important for determining the degree of catechin separation and their identification.

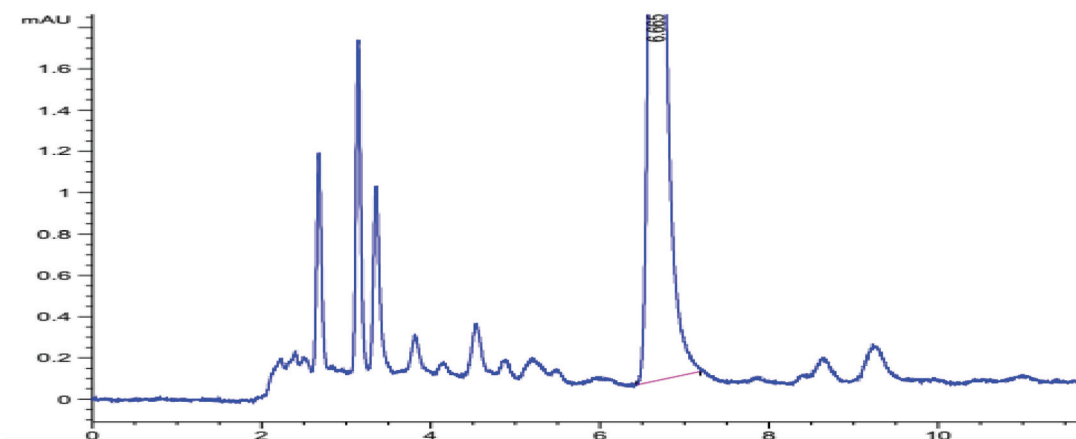
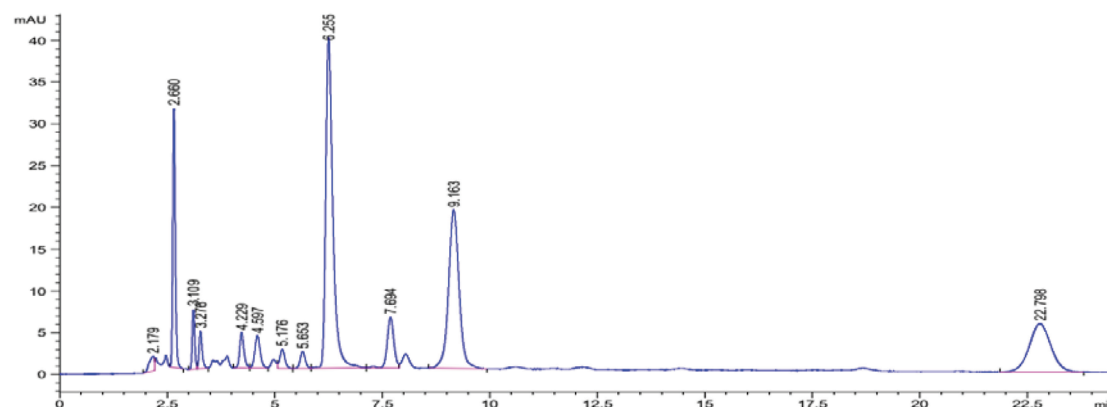


Fig.3. *Chromatographic profile of black tea*

Fig. 3 shows the chromatographic profiles of black tea catechins. Acetonitrile water 1% acetic acid 15:80 was used as the eluent. Chromatograms show that the concentrations of epigallocatechin gallate as well as galocatechin, epigallocatechin

and epicatechin are low in black tea extract. At the same time increased peaks, which according to time constraints and literary data [22] can be considered as representatives of the Teaflavins and Thearubigins (Fig. 3, peaks from left 2 and 3).



(A)

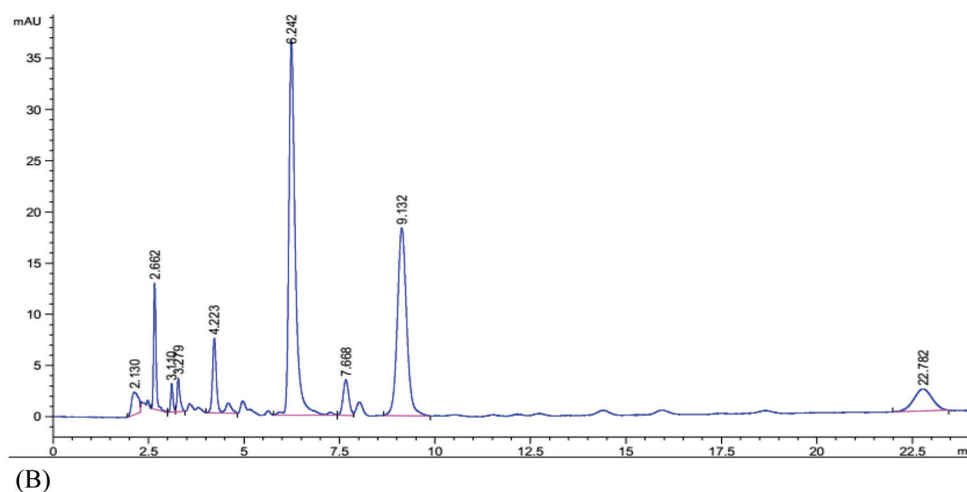


Fig. 4. Commercial green tea chromatograms: A - "Greenfield"; B - "Shemokmedi"

The chromatograms shown in Figure 4 reveal the four major peaks of catechin by time retention (min): 4.2 - epigallocatechin; 7,6 - epicatechin; 9.1 – epigallocatechin gallate and 22.7 – epicatechingallate.

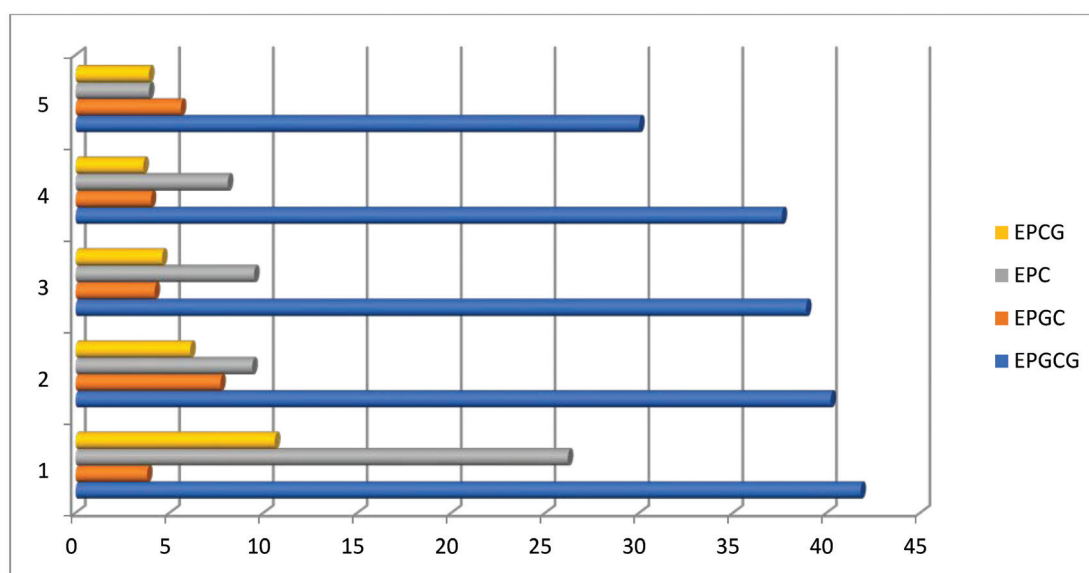


Fig.5. Comparative amount of four catechins in commercial green tea extracts: "Greenfield"; 2- "Shemokmedi"; 3 - "Richards"; 4 - "Akhmad"; 5 - "Gurieli"

Based on the results of quantification of catechins, the high content of epigallocatechin gallate in commercial green tea extracts "Greenfield" and "Shemokmedi" has also been verified (Fig. 5). On the other hand, compared to imported green tea, Georgian commercial tea "Shemokmedi" and "Gurieli" contain more epigallocatechin. It is noteworthy that the epigallocatechin, like the epigallocatechin-gallate, is also characterized by high biological activity, as evidenced by the literature [14, 15].

The study results of experimental tea extracts show high rates of red tea in terms of total phenols, antioxidant activity and individual catechins (Fig. 6), which is due to the use of a new technological process for making this type of tea.

It is planned to continue the work of creating and researching new types of tea products with high catechin substitution, high antioxidant activity, low caffeine and distinctive taste properties in the future.

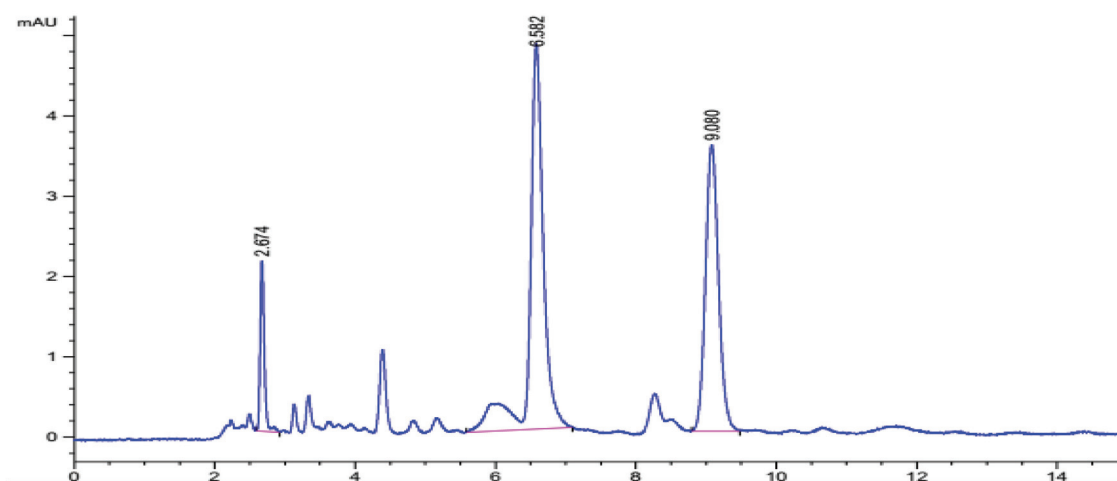


Fig. 6. *Chromatographic profiles of experimental red tea samples*

Conclusion

1. The best eluent solvents under the stationary phase conditions of the high performance chromatographic method are selected. Detection of compounds at 278 and 210 nm under two different wave conditions allows to increase catechin peaks and more accurately identify these substances.

2. Catechins and antioxidant activities of 12 different types and origins of experimental and commercial tea extracts have been studied by using a high pressure chromatographic method. Particular catechins are examined in detail: epigallocatechin-gallate, epigallocatechin, epicatechin and epicatechingallate.

3. High rates of experimental and commercial tea products of Georgian population with antioxidant activity and catechins are established - According to the amount of epigallocatechin gallate and epigallocatechin. the high substitution of epigallocatechingallate in the commercial Georgian green tea extract “Shemokmedi” is worth emphasizing.

4. Along with the distinctive taste properties, high rates of experimental red tea have been shown in terms of total phenolic compounds, antioxidant activity and individual catechins, resulting from instantaneous and deep inactivation of oxidizing enzymes in the technological process of making this product.

The manuscript is dedicated memory of the late Professor Mikhail Zaprometov, who was a prominent scientist in the field of biochemistry of phenolic compounds. It was during the study of the biochemical study of Georgian tea, that Mikhail Zaprometov obtained the first crystalline compounds of Georgian

tea catechins in 1952, which gave scientists even more incentive and opportunity for research. The idea of working on tea catechins arose during the celebration of the 100 th anniversary of the birth of Mikhail Zaprometov, the memory of which is dedicated to the present article.

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References

- [1] M.N. Zaprometov, Biochemistry of Catechins, Nauka, Moscow, 1964 (in Russian).
- [2] Bernatoniene J, Kopustinskiene DM., The Role of Catechins in Cellular Responses to Oxidative Stress. *Molecules*, 20, 23 (4) 2018. pii: E965. doi: 10.3390/ molecules 23040965..
- [3] Peluso I, Serafini M., Antioxidants from black and green tea: from dietary modulation of oxidative stress to pharmacological mechanisms. *Br J Pharmacol.* 174 (11) (2017) 1195-1208.
- [4] Ohishi T, Goto S, Monira P, Isemura M, Nakamura Y., Anti-inflammatory Action of Green Tea. *Antiinflamm Antiallergy Agents Med. Chem.*, 2016; 15(2):74-90. doi: 10.2174/1871523015666160915154443.
- [5] Reygaert WC1., Green Tea Catechins: Their Use in Treating and Preventing Infectious Diseases. *Biomed Res Int.* 2018 Jul 17; 2018:9105261. doi: 10.1155/2018/9105261. eCollection 2018.
- [6] Jun Xu , Zhao Xu and Wenming Zheng, Review of the Antiviral Role of Green Tea Catechins *Molecules* 2017, 22, 1337; doi:10.3390/ molecules22081337.

- [7] Musial C, Kuban-Jankowska A, Gorska-Ponikowska M., Beneficial Properties of Green Tea Catechins. *Int J Mol Sci.* 2020 Mar 4;21(5). pii: E1744. doi: 10.3390/ijms21051744.
- [8] Green tea effects on cognition, mood and human brain function: A systematic review. Mancini E, Beglinger C, Drewe J, Zanchi D, Lang UE, Borgwardt S. *Phytomedicine.* 2017 Oct 15;34:26-37. doi: 10.1016/j.phymed.2017.07.008. Epub 2017 Jul 27. Review.
- [9] Khalesi S, Sun J, Buys N, Jamshidi A, Nikbakht-Nasrabadi E, Khosravi-Boroujeni H., Green tea catechins and blood pressure: a systematic review and meta-analysis of randomised controlled trials. *Eur J Nutr.*, 53(6) (2014) 1299-311. doi: 10.1007/s00394-014-0720-1. Epub 2014 May 27.
- [10] Momose Y, Maeda-Yamamoto M, Nabetani H., Systematic review of green tea epigallocatechin gallate in reducing low-density lipoprotein cholesterol levels of humans. *Int J Food Sci Nutr.*, 67(6) (2016) 606-13. doi: 10.1080/09637486.2016.1196655. Epub 2016 Jun 20.
- [11] Cicero AFG, Colletti A., Polyphenols Effect on Circulating Lipids and Lipoproteins: From Biochemistry to Clinical Evidence. *Curr Pharm Des.* 2018;24(2):178-190. doi: 10.2174/1381612824666171128110408.
- [12] Kim SN, Kwon HJ, Akindehin S, Jeong HW, Lee YH., Effects of Epigallocatechin-3-Gallate on Autophagic Lipolysis in Adipocytes, *Nutrients*, 30, 9(7) (2017). pii: E680. doi:10.3390/nu9070680.
- [13] Vázquez Cisneros LC1, López-Uriarte P, López-Espinoza A, Navarro Meza M, Espinoza-Gallardo AC, Guzmán Aburto MB., Effects of green tea and its epigallocatechin (EGCG) content on body weight and fat mass in humans: a systematic review. *Nutr Hosp.*, 5;34(3) (2017) 731-737. doi: 10.20960/nh.753.[Article in Spanish; Abstract available in Spanish from the publisher].
- [14] Kim HS, Moon JH, Kim YM, Huh JY., Epigallocatechin Exerts Anti-Obesity Effect in Brown Adipose Tissue. *Chem Biodivers.* 2019 Oct;16(10):e1900347. doi: 10.1002/cbdv.201900347. Epub 2019 Sep 18.
- [15] Tarakhovskiy Y., Cim Y., Abdrasilov B., Musafarov E., *Flavonoids: biochemistry, biophysics, medicine*, Sinkrobuk, Puskino, 2013 (in Russian).
- [16] Chkhikvishvili I., *Flavonoids. Biochemistry, Food and Health*, Tsodna, Tbilisi, 2010 (in Georgian).
- [17] Zaiter A., Becker L., Karam M-C., Dicko A., Effect of particle size on antioxidant activity and catechin content of green tea powders. *J. Food Sci Technol.*, 53(4) (2016)2025–2032 DOI 10.1007/s13197-016-2201-4
- [18] ISO 14502-2. ,Determination of substances characteristic of green and black tea-Part 2: Content of catechins in green tea - Method using high-performance liquid chromatography, 2005.
- [19] ISO14502-1. Determination of substances characteristic of green and black tea-Part1: Content of total polyphenols in tea – Colorimetric method using Folin-Ciocalteu reagent, 2005.
- [20] Kupina S, Fields C, Roman MC, Brunelle SL. Determination of Total Phenolic Content Using the Folin-C Assay: Single-Laboratory Validation, First Action 2017.13. *J AOAC Int.*, 1;101(5) (2018) 1466-1472. doi: 10.5740/jaoacint.18-0031. Epub 2018 Jun 12.
- [21] Vaishali Sharma, Ashu Gulati, Srigurupuram Desikachary Ravindranath, Vipin Kumar. A simple and convenient method for analysis of tea biochemicals by reverse phase HPLC, *J. of Food Composition and Analysis*, Vol. 18, Issue 6 (2005) 583-594.
- [22] Revishvili T., Apkhazava D., Khomeriki M. Technological process of receiving preparations biological active compounds of tea. In: *International Scientific Conference “Modern technologies to produce ecologically pure products for sustainable development of Agriculture”*. Tbilisi, Georgia, on 28-30 September 2016. pp. 598-600 (in Georgian).
- [23] Deng S, Jin J, He Q. Inhibitory Effect of Epigallocatechin Gallate, Epigallocatechin, and Gallic Acid on the Formation of N-Nitrosodiethylamine In Vitro. *J Food Sci.*, 84(8) (2019) 2159-2164. doi: 10.1111/1750-3841.14737. Epub 2019 Jul 22.