Exploring the potential of total polyphenols in the leaves of berry plants for a healthy diet

Konstantin Martynenko[1], *Petr* Balanov[1], *Irina* Smotraeva[1], and *Olga I*vanchenko[2]

[1]Faculty of Biotechnologies (BioTech), ITMO University, 49 Kronverksky Ave, Saint Petersburg, 197101, Russia
[2]Peter the Great St. Petersburg Polytechnic University (SPbPU), 29 Politekhnicheskaya St., St. Petersburg,195251, Russia

Abstract. Today, most researchers believe that polyphenols have wide-ranging applications and protect the human body from many diseases. This paper studies the total phenolic content (TPC) and the content of heavy metals in the selected leaves of plants that athletes actively use as a supplement to a healthy diet. For analysis, blackberry, raspberry, currant, and hawthorn leaves are used, and green tea, black tea, and chamomile flowers are used for comparison of characteristics. The extractive and total content of phenols in the vegetative part of berry crops are the most favorable indicators for fortifying sports nutrition products because these indicators are comparable to that of green and black tea. Berries and fruits have small antioxidant capacities, since they have high sugar content. Sugar provides high extractability but does not contribute to antioxidant effectiveness.

1 Introduction

There are numerous scientific publications on the antioxidant effect of plant foods and the associated positive effects on the prevention of chronic diseases. Many surveys have been conducted, but a very small part of them studied vegetative parts of plants, the berries and fruits of which are actively used in the Russian food industry. Concerning the composition of the antioxidant-active components of these products, the same situation is reported [1,2].

Modern studies show that high activity of athlete does not guarantee preventing the further development of atherosclerosis. But recent evidence shows that flavonoids and anthocyanins of polyphenols group, which can be found in berry plants, protect the body's cells from free radicals and inhibit cellular oxidation. Most importantly, they can reduce plaques in blood vessels and thus prevent atherosclerosis. For example, a human study showed that after a year-long consumption of pomegranates, the thickness of the internal vascular wall of the carotid artery decreased by 30% in patients with atherosclerosis, while it increased by 9% in the control group. This improves not only the blood flow but also vascular wall elasticity [3].

A study by the Vanderbilt University Medical Center showed that regular consumption of fruit and vegetable juices (a potential source of antioxidant polyphenols) can reduce the risk of Alzheimer's disease by up to 76 % [4].

However, it is better to consume raspberry, blackberry, or currant leaf tea rather than fruit juice to counteract high fructose levels. This is because berries contain fewer polyphenols than the leaves of these plants.

Additionally, various studies with pomegranate have shown that the growth of breast, lung, skin, and prostate cancer cells have been inhibited by polyphenols [5].

The polyphenol taxifolin from deciduous plant extracts is often used to treat cerebral infarcts and their sequelae [6].

Polyphenols from grapes can prevent plaque and biofilm formation on teeth by inhibiting the bacterial species Streptococcus mutans. Thus, they also have a preventive effect on dental caries [7].

Biologically active substances (BAS) from plant raw materials have recently become the center of sports medicine. For athletes, a healthy gut is particularly important in many ways. It is the largest human immune organ and has the most efficient absorption of micronutrients and macronutrients. Polyphenols from fruits, berries, and tea interact with the microbiome, are metabolized, and have numerous antioxidant, anti-inflammatory, and immunomodulatory effects. Flavonoids and other bioactive substances from fruit teas also reduce exercise-related oxidative stress and thus they damage the development of muscle tissue. These are just a few of the positive effects of BAS on athletes' performance.

Natural polyphenols are found in plants in the form of bioactive substances such as dyes, flavorings, and tannins. They are designed to protect the plant from predators and to attract insects to pollinate it with bright colors. While flavonoids give plants the yellow color, anthocyanins are responsible for red, blue, and purple coloration. Polyphenols serve to protect the plant from pathogens and disease, they are found mainly in the rind and in the vegetative parts of plants.

Studies show that a diet high in polyphenols provides protection against inflammation caused by heavy muscular exertion. The products with polyphenols, being introduced into sports nutrition, can help reduce inflammatory responses caused by exercises. Women benefit from the antioxidant action of polyphenols, since they have an increased sensitivity to oxidative stress due to decreased estrogen production.

This paper presents data on the total content of phenols and the percentage of extractive substances in the vegetative parts of plants based on laboratory studies. Comparative analysis, quantification, and isolation of selected samples for their antioxidant activity are to be carried out [8,9].

2 Materials and methods

Dried plant (blackberry, raspberries, black and green tea) leaves, fresh berries and flowers (hawthorn and chamomile) were used in the study. For analysis, a water extract was prepared for 10 minutes. To make the extraction of substances into the solution complete it is necessary to add methyl alcohol [10].

2.1 Total Phenolic Content (TPC) activity

The total phenolic content was determined using the Folin–Ciocalteu reagent. This method is based on the reducing ability of phenolic compounds. Easily oxidizable substances such as polyphenols or ascorbic acid can reduce complexes. The complexes are formed in a mixture of tungsten blue oxide (W_8O_{23}) and molybdenum oxide (Mo_8O_{23}) to blue pigments. The intensity of the resulting color, which is proportional to the content of oxidized substances, is determined photometrically. Various phenolic compounds react with metal complexes of different sensitivities. The reactivity of the compound increases proportionally to the amount of free phenolic hydroxyl groups. In this case, two OH groups

in the ortho- or para-position to each other increase the reactivity. These features are also thought to be responsible for the antioxidant effect of polyphenols [11].

2.2 Determination of water-soluble extractives

The method is based on the extraction of water-soluble substances from the sample. The first stage includes boiling with a reverse refrigerator, the second stage includes assessment of the dried extract.

The mass fraction of the aqueous extract (X1, % per dry substance) is found by the formula:

$$X_1 = m_1 \frac{(500 * 100 * 100)}{50 * m_0 * R_s}$$

where m_1 is the mass of dry aqueous extract, g;
m_0 is the mass of tea sample, g;
R_s is the mass fraction of dry substances of tea, %.

2.3 Determination of the mass fraction of dry substances

The method is based on drying a sample to a constant weight at 103±2 °C. Dry residue as a percentage of the original sample gives the content of dry matter in the test sample. Drying to a constant weight is carried out in glass beakers.

Mass fraction of solids is calculated by the formula:

$$R_s = \frac{(m_2 - m_0)}{(m_1 - m_0)} * 100$$

where m_2 is the mass of the sample buster after drying, g;
m_0 is the mass of empty sample buster, g;
m_1 is the mass of the buster with a sample before drying, g;
The result of the analysis is the arithmetic mean of two parallel definitions. A discrepancy between them of about 0.3% is allowed.

2.4 Analysis of heavy metals in plants

The raw materials were assessed using the MAX-GF2E spectrometer. A spectrometer is the analytical equipment designed to determine the content of chemical elements in a solid, powdery, or dissolved state. The spectrometer operation principle is based on the excitation of chemical elements and the registration of their radiation. The intensity depends on the quantitative content of these elements in the analyzed object. The excitation source in the MAX-GF2 spectrometer is a low-power X-ray tube generating electromagnetic radiation in the X-ray range [12, 13].

3. Results

Table 1 shows the content of total polyphenols and water-soluble extractives in samples based on a weight of 2 g/200 ml with infusion time of 10 minutes. The total content of phenols in aqueous infusions is presented in terms of gallic acid. The obtained data showed

that the number of extractive substances and the total content of phenols approximately correlate with each other, even it is impossible to establish a completely linear dependence. This is an expected result, since the unequal properties of analytes by both measurement methods were used. However, it can be said that samples with a high content of phenols also show a high number of water-soluble extractives and vice versa.

Table 1. Content of total polyphenols and water-soluble extractives

	General content of phenols, % of dry matter	Extractives, % of dry matter
Green tea (leaf)	16.24	52.34
Blackberry (leaf)	16.22	46.86
Raspberry (leaf)	12.32	38.92
Currant (leaf)	10.86	35.46
Black Tea (leaf)	10.78	42.46
Hawthorn (fruit)	9.34	29.88
Chamomile (inflorescences)	6.64	18.48

As expected, green tea has the highest contents of phenols and extractives. The leaves of blackberries, raspberries, black tea have smaller contents, and the lowest contents were registered in fruits and flowers of hawthorn and chamomile. The leaves of blackberry, raspberry, and currant showed excellent results. These plants have the most favorable ratio of extractability and total phenolic content. The least effective is the potential of extract in berries. This may partly be due to the high sugar content in these samples. Sugar provides high extractability but does not contribute to the antioxidant effect; it has regenerated properties. The total contents of polyphenols registered in green and black tea are somewhat lower than those described in the literature. This can be explained by the variety of origin, the way of gathering the tea and in the type of extraction.

All samples were also analyzed for safety. The results of tests for heavy metals are presented in Table 2.

Table 2. Examination of samples for heavy metals

	As, mg/kg	Zn, mg/kg	Sr, mg/kg	Fe_2O_3,%	MnO, mg/kg	Cr, mg/kg	TiO_2, %
Green tea	5	65	94	0.02	330	27	0.05
Bramble	2	59	72	0.00	127	28	0.04
Raspberry	4	50	85	0.03	851	32	0.05
Currant	3	40	111	0.06	229	29	0.04
Black tea	2	60	87	0.04	442	54	0.06
Hawthorn	1	76	60	0.07	594	43	0.03
Chamomile	4	45	65	0.03	543	34	0.02

The low level of heavy metals indicates the safety of the raw material. Due to their physical structure, plants cannot avoid adverse environmental conditions, so heavy metals can accumulate in large quantities. To guarantee their existence since their evolution millions of years ago, plants have had to develop many strategies to combat natural enemies, seasonal fluctuations, and climatic changes.

4. Conclusion

The studies of common polyphenols in leaves can make a positive contribution to the antioxidant activity of human diet. In this work, assessed was the content of common polyphenols in plant leaves, which can be used not only as an additive to green tea, but also as a healthy food. Blackberry leaves, raspberries, currants, hawthorn berries, chamomile flowers were studied, and their characteristics were compared to those of green and black tea. The levels of extractive substances ranged from 18.48% (Chamomile) to 52.34% (green tea). The total content of polyphenols varied from 6.64% (Chamomile) to 16.24% (green tea).

The highest number of polyphenols has the green tea. Blackberry leaves contained 5.44% more polyphenolic compounds than black tea (10.78%) and less than green tea (16.24%) by an insignificant percentage. Raspberry leaves (12.32%) and black tea (10.78%) indicators had no significant differences, so they can be used interchangeably in nutrition. Blackberry and raspberry leaves have a similar composition due to their belonging to the same plant family.

The obtained data state that the high content of polyphenols and high extractability of the infusions indicate a good potential of this raw material for the food industry. The relevance of the use of herbal and fruit teas is the lack of physiological caloric content, but juices and wines contribute to overeating. The negative consequences caused by alcohol consumption are also eliminated.

References

1. D.P. Makris, G. Boskou, Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. Journal of Food Composition and Analysis, **20 (2)**, 132. (2007)
2. Y.S. Lin, , Y.J. Tsai, Factors affecting the levels of tea polyphenols and caffeine in tea leaves. Journal of Agricultural and Food Chemistry, **51 (7)**, 1864-1873 (2003)
3. M. Aviram u. a.: Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. In: Clinical Nutrition. **23**, 423–433 (2004)
4. Qi Dai, MD, PhD, Amy R. Borenstein, PhD, Yougui Wu, PhD, James C. Jackson, PsyD and Eric B. Larson, MD, MPH: Fruit and Vegetable Juices and Alzheimer's Disease: The Kame Project. In: Am J Med. **119(9)**, 751–759 (2006)
5. E. P. Lansky, R. A. Newman: Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. In: J Ethnopharmacol. **109(2)**, 177–206 (2007)
6. Adeleh Esfandiari, A. Paul Kelly: The effects of tea polyphenolic compounds on hair loss among rodents. Journal of the National Medical Association. **97(8),** 5 (2005).
7. K. Hyun, Chemical Characterization of Red Wine Grape and Pomace Phenolic Extracts. Journal of Agric Food Chem. **55 (25)**, 10200–10207 (2007)
8 C. Astill, M.R. Birch, Factors affecting the caffeine and polyphenol contents of black and green tea infusions. Journal of Agricultural and Food Chemistry, **49 (11)**, 5340–5347 (2001)
9 J. Wollgast, E. Anklam, Review on polyphenols in Theobroma cacao. Food Research International, **33 (6)**, 423–447 (2000)
10. J. Azmir, I.S.M. Zaidul, Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering, **117 (4)**, 426–436 (2013)

11 M.P. Kahkonen, A.I. Hopia, Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry, **47 (10)**, 3954–3962 (1999)

12 K. Jurowski, M. Folta, B. Tatar, The Toxicological Risk Assessment of Cu, Mn, and Zn in Herbal Medicinal Products. Journal of Biological Trace Element Research, **200 (4)**, 1949–1955 (2022)

13 X. W. Wang, H.Y. Liu, X.F. Gu, Y. Tu, E. J. Yu, Distribution Characteristics of Heavy Metals in Soils Affected by Different Land Use Types in a Superimposed Pollution Area with High Geological Background. Journal of Huanjing Kexue/Environmental Science, **43 (4)**, 2094–2103 (2022)

14 E.W.C. Chan, E.Y. Soh, P.P. Tie, Antioxidant and antibacterial properties of green, black, and herbal teas of Camellia sinensis, Journal of Pharmacognosy Research, **3 (4)**, 266–272 (2011)

15 J. Oh, H. Jo, A.R. Cho, S.J. Kim, J. Han, Antioxidant and antimicrobial activities of various leafy herbal teas. Journal of Food Control, **31 (2)**, 403–409 (2013)

16 E. Shannon, A.K. Jaiswal, N. Abu-Ghannam, Polyphenolic content and antioxidant capacity of white, green, black, and herbal teas: A kinetic study. Journal of Food Research, **2 (1)**, 1–11 (2018)