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The chemical complex of Caucasian blackberry (*Rubus caucasicus* L) leaves

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Abstract
Studied some physical and chemical characteristics and 6-sheet blackberry shoot Caucasian (*Rubus caucasicus* L.). It was found that the minima of the moisture content, the extract substances and phenolic compounds to the same period of the beginning and end of the growing season of plants. The composition of phenolic compounds represented by catechins, flavonols and leuco anthocyanidins. Highs accumulation of phenolic compounds during the growing season are found in the middle of the season (July-August).
The amount of free amino acids blackberry leaf vegetation monthly averages 26.68 mg/g. 11 of the identified individual amino acids are essential 5 (His., Arg., Met., Leuc., Val.,). The complex oxidative enzymes blackberry leaf established the presence of the active form of O-diphenol oxidase. Blackberry leaf and extract has high antioxidant activity.

Keywords: Herbal teas, Caucasian blackberry, physical and chemical composition, phenolic substances, amino acids, oxidative enzymes, antioxidant activity.

Introduction
Based on the analysis of data on napitochnyh plants used for the preparation of herbal teas, taking into account available resources and people's experience of their application, we have as the object of study chosen blackberry leaves caucasian.
Blackberries belong to the subgenus *Eubatus*, genus Rubus L. of the family Rosaceae, 50-150 cm tall shrub, with long rhizome and perennial aboveground stems, covered with spines. In the Caucasus, found 33 species.
From these kinds of blackberries in Georgia is mainly spread Caucasian blackberry (*Rubus caucasicum* L.)
The plant blooms from May to August, fruiting after about 1.5 months from flowering. It grows in woods, ravines, among shrubs, clearings, along the banks of rivers, streams, meadows, rocky slopes, orchards, gardens, roadsides. It forms large thickets. The total area of blackberries in Georgia up to 300 thousand. Ha.
A distinctive feature of Caucasian blackberry brambles from other species is a little prickly education stems and leaves, which facilitates the collection of raw materials.
What is essential is that the periods of vegetation Blackberry coincide with periods of tea production season (April-October months), which is of great importance in the industrial development of its production in enterprises of primary processing of tea.
It should be noted that the information on the chemical composition of the plants are scarce, except for works relating to the study of the texture of a 6-sheet escape blackberry and individual phenolic compounds flush elements [25, 28].
The study of these questions will determine the purpose of the study.

Materials and Methods
The objects of study were Caucasian blackberry leaves. The raw materials for the growing season were studied: phenolic compounds - by Leventhal using conversion factor K = 4,16, free amino acids – Method chromatography paper [35] and flavonoids – by fractionating the total preparation [14, 15, 32, 36], protein substances – by micromethod [33], vitamin C -by [33], ash elements– by method of wet combustion [33], certain trace elements – by using a flame photometer [33], antioxidant activity-by method of Ferric Reducing Ability of Plasma (FRAP) [34].

Research and Their Discussion

Phenolic and amino acid composition

In the production of tea phenolics play a primary role, as their ability to be oxidized by enzymes to form a red-brown and the reaction products, determined by inherent characteristics of the finished product quality. Phenolic compounds in the manufacture of tea leaf tea undergo a deep and diverse transformations that form the basis of the process of tea production. Therefore it is clear the enormous work carried out by researchers in the study of the role and significance of tea phenolic compounds [1-6, 12-13, 18-23, 37, 40, 56-49].

In connection with the above, in the production of herbal tea is paramount study phenolic compounds used or that the vegetable raw materials.

Especially interesting is the establishment of seasonal dynamics of phenolic compounds during the growing season of the plant. As is known, the content of phenolic compounds in tea leaves subject to change during the processing season - for the Georgian tea plant the maximum of their savings in other similar conditions the same as the middle of the season (July-August), when the daily total atmospheric temperature is the highest. Details of the experiment conducted by us in blackberry escape during the growing season (Table 1)

As a result, it was found that the nature of the accumulation of phenolic compounds blackberry escape by month growing season is identical to the tea leaves. The BlackBerry, as well as in tea leaves maximum accumulation of phenolic substances comes to the middle of the growing season - the hottest period (July-August). This once again confirms that the sheet of blackberry, as well as tea and other plant materials. Fully complies to the basic laws of ontogeny of plants.

Table 1: Dynamics of phenolics blackberry escape during the growing season

S. No	Months	Phenolic compounds, % of dry weight	The extent of the maximum accumulation of phenolic compounds in%
1.	May	16,70±0,24	82,1
2.	Jun	18,84±0,22	82,5
3.	Jul	19,61±0,33	96,4
4.	August	20,35±0,37	100,0
5.	September	16,65±0,21	81,8
	Average	18,43±0,30	

In further work our first important issue is the study of the qualitative composition of phenolic compounds blackberry leaf. As on our previous work [26] has been established qualitative composition of phenolic compounds blackberry leaf consisting of three groups of flavonoids: leukoanthocyanidins, catechins and flavonols, which are two-dimensional chromatograms are shown in Figure 1.

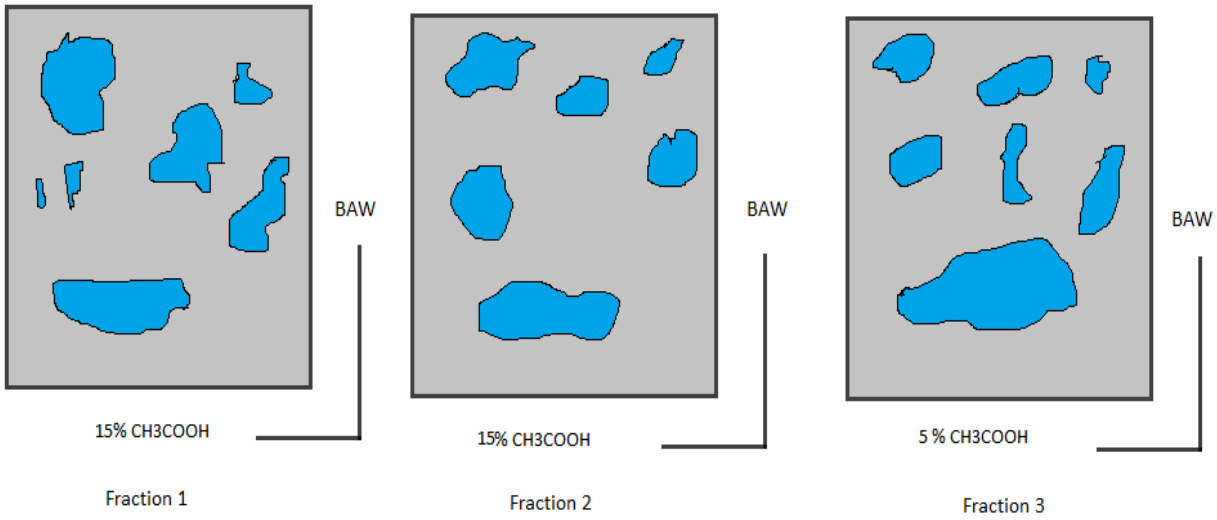

Fig 1: A two-dimensional chromatogram of a fraction of the total drug flavonoids blackberry leaf

- direction –BAW (n- butane-acetic acid -water 4:1:5; the upper phase);
- direction – a 15% acetic acid (lower phase)
- In the study of the dynamics of accumulation of flavonoid compounds in blackberry escape during the growing season revealed that after the flowering phase (month of May) the amount of catechins and flavonols leukoanthocyanidins increases during the summer, peaking in August and then decreases (Fig 2-4).

The dynamics of flavonoids in blackberry escape and quantitative changes in vegetation indicates that these substances are actively involved in the metabolic processes of the plant cell [8, 11, 14, 16, 22].

It is known that many of the chemicals in the manufacture of tea leaf tea plays an important role proteins and amino acids. The basis of the structure of proteins is a complex combination of different amino acids.

The value of proteins in the formation of quality of tea is, in addition to their participation in the enzymatic reaction in that they are a source of amino acids in the processing of the sheet a partial their hydrolysis and formed with amino acids, according to available data [15] are directly involved in formation of tea aroma.

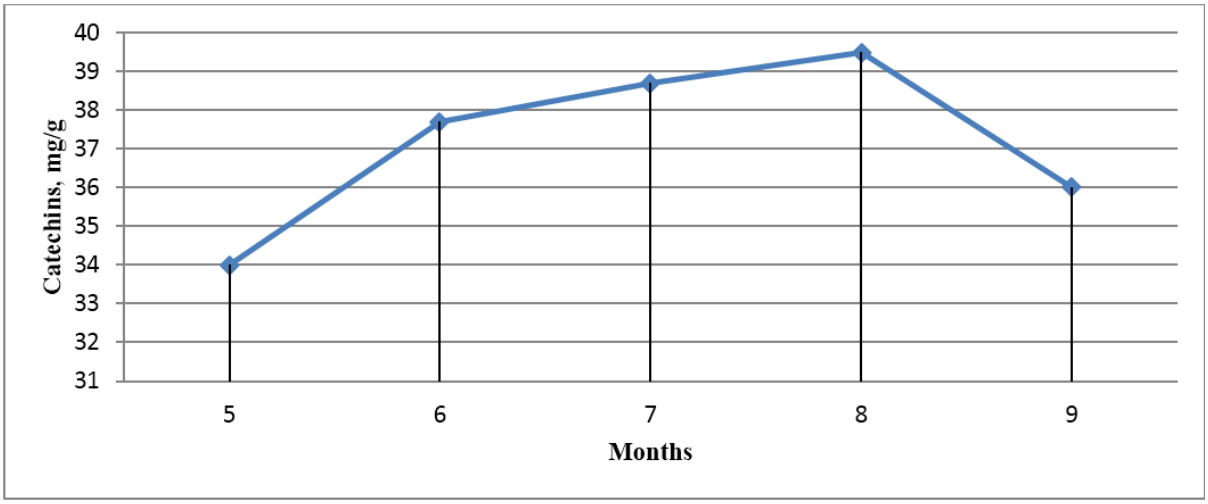

Fig 2: Dynamics of catechins blackberry escape during the growing season

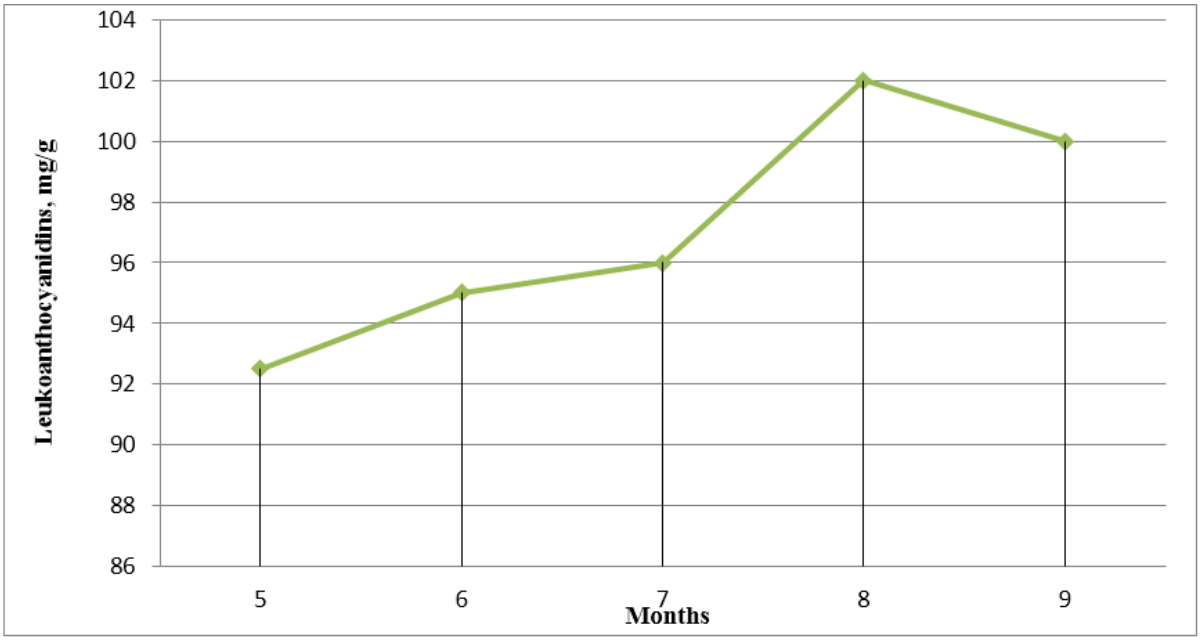

Fig 3: Dynamics leukoanthocyanidins in blackberry escape during the growing season

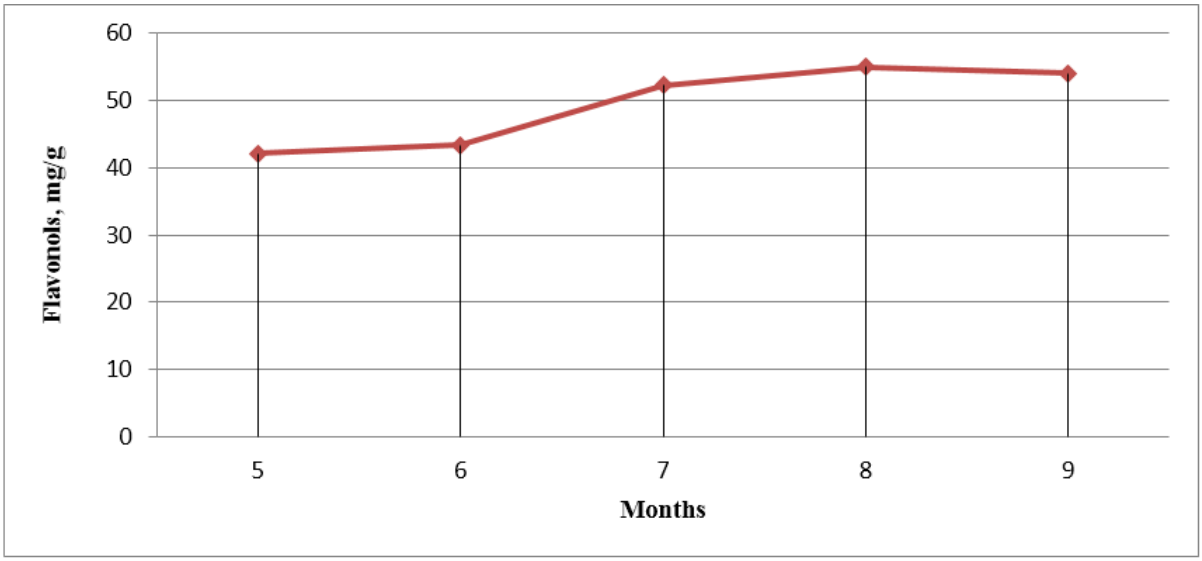

Fig 4: Dynamics of flavonols in blackberry escape during the growing season

Studies conducted by Soviet and foreign scholars on the study of the amino acid composition of tea leaves, are found in the 17 individual amino acids [7, 41].

Given the importance of proteins and amino acids in the production of tea, we conducted studies on them as part of the blackberry leaf. Quantitative determination of proteins was carried out on the content of total nitrogen using micro-method Keldal [34, 33].

At the same time also carried out a quantitative determination of the total amount of free amino acids in the context of the seasonal vegetation 6-leaf blackberry shoot (Table 2).

Table 2: The content of proteins and free amino acids in the blackberry escape during the growing season

S. No.	Months	The protein substance, % on dry basis		The amount of free amino acids, g / 100g
		Total nitrogen	Proteins	
1.	May	0,381	2,381	26,15
2.	Jun	0,364	2,275	26,56
3.	Jul	0,360	2,250	27,47
4.	August	0,360	2,250	27,82
5.	September	0,372	2,325	25,40
Average during the growing season		0,367	2,296	26,68

As you can see, the protein content in the beginning of the growing season maximum, then gradually decreases and lows is in July-August. In these months the maximum accumulation of free amino acids.

Of particular interest is the study of individual amino acids in the proteins of the blackberry leaf. Using the method of paper chromatography, we isolated 11 individual amino acids and established their quantitative content (Table 3).

As can be seen, blackberry leaf is characterized by high-quality composition of individual amino acids: 11 amino acids identified by us 5 are "indispensable" (histidine, arginine, methionine, leucine, valine).

Table 3: The content of individual amino acids in the blackberry escape

S. No	Amino acids	Amino acid, mg / g dry matter
1.	Cysteine	0,92
2.	Lysine	1,67
3.	Histidine	2,63
4.	Asparagine	2,66
5.	Arginine	3,43
6.	Glutamic acid	3,01
7.	Tyrosine	4,02
8.	Methionine	2,21
9.	Leucine	2,55
10.	Phenylalanine	2,53
11.	Valine	0,85
Total		26,70

Valine in both plants contained approximately equal amounts (0.85 mg/g blackberry leaf and 0.68 mg/g - in tea). Simultaneously, blackberry leaf contained 2.21 mg/g of methionine, in which tea leaf is detected.

As the total amount of amino acids blackberry leaf tea is much superior (26.7 mg/g against 17.45 mg/g), while the total content of essential amino acids, this superiority is expressed in the 9.61 mg/g (11.67 mg/g blackberry sheet and 2.06 mg/g - in the tea). If we consider that of the essential amino acid histidine is essential for normal growth of infants [16] and take into account the lack of blackberry leaf potent alkaloids, including caffeine, it creates a good foundation for the widespread use of blackberry tea as a dietary and baby food.

Oxidative enzymes

Enzymes, mainly oxidation, underlying biochemical processes of tea production, causing major biochemical transformations that favor formation of specific flavors and aromas of the finished product. Study of the enzymes in the tea leaf tea and ready the subject of numerous works [1, 9-10, 17, 24, 32-33, 35-36, 39-45, 47]. These studies found that tea leaves are present in very active oxidizing enzymes -0-difenoloksidaza and peroxidase. It was also found that the main oxidation processes in both tea leaf during growth and development and during processing, performed these enzymes [1, 6, 33, 36, 47] with which the respective substrates are transformed *in vivo* tea leaf and during its processing.

Given the above, we conducted a study of oxidative enzymes in the manufacture of blackberry leaf herbal tea from it. At the heart of the experiments lay obtain acetone extract of the blackberry leaf, and a study of its activity against tea tannin and phenolic substances blackberry.

For preparation of 500 g of acetone blackberry fresh shoots were placed in a special grating device having a jacket for liquid nitrogen and provided with a sharp rotating blade (7-8 th. rev./min.). In this device material is quickly frozen and pulverized in this state to complete destruction of tissues (3-5 min.). The milled sheet was treated in a homogenizer 2-3 times with a 1 minute cold (-15 ^{0}C) 80% aqueous acetone. Each time the suspension was filtered through a Buchner funnel and the precipitate washed with a small amount of dry acetone. The precipitate was dried at room temperature in a stream of air.

The activity of acetone extract of the blackberry leaf tea tannin was determined to ampermetrical method on polarography. Reaction medium: 10 g of acetone and 1 ml of the preparation of 0.1 M citrate-phosphate buffer pH 5, 7. to this reaction mixture was added 20 .mu.l micropipette 0.35M tea tannin aqueous solution (312 mg in 2 mL water) with a molecular weight of 450.

Polarographic cell pre-incubated at 25 ^{0}C. Next calculated activity per 1 mg of acetone extract. She was 20 n.mol O2 / min.mg.

Control reaction medium was the same, only the polarographic cell was pretreated in an oven at 1000C preparation acetone for 1 hour. The activity of acetone extract of the blackberry leaf to the phenolic substances blackberries also determined by ampermetrical on polarograph and was as follows: the reaction medium - 10 mg acetone drug blackberry leaf, 1 ml of 0.1 M citrate-phosphate buffer pH 5, 7. To this reaction medium were added 20 l of an aqueous solution (312 mg phenolic substance, dissolved in 2 ml of water), the temperature of the medium 25 ^{0}C.

After calculation of the activity was 25 n.mol/min.mg per 1 mg of acetone extract. Control reaction medium was similar to the above-mentioned.

The study of the activity of the drug in the acetone tea tannin was also carried out in the Warburg apparatus. Reaction medium: 25 mg of blackberry leaf drug acetone, 2.3 ml of 0.1 M citrate-phosphate buffer pH 5,7, 126 mg tea tannin dissolved in 1.75 ml of water to obtain tannin 0.16M, 0.5 ml of which was added to the reaction medium. In the center apparatus Warburg was added 0.2 ml of 5 M NaOH.

The report was carried out every 15 minutes. The dishes were thermobarometers same reaction medium, only acetone drug pretreated in an oven at 100 ^{0}C for 1 hour to completely inactivate the enzyme preparation acetone.

Figure 5. Shows activity data acetone extract from the leaves of the blackberry against oxidation tea tannin.

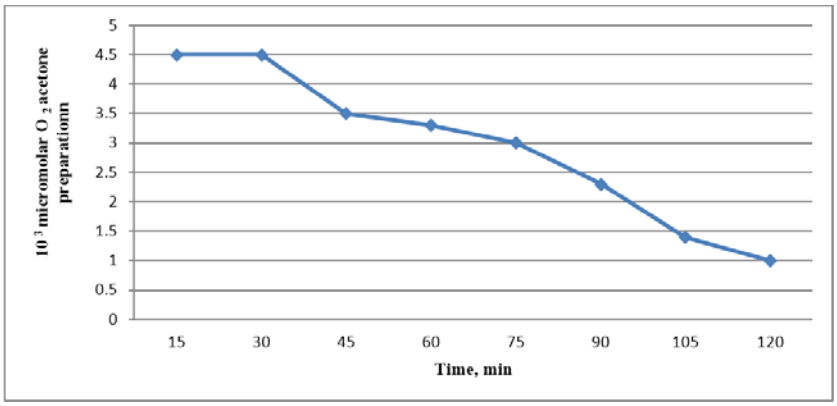

Fig 5: The activity of acetone extract of leaves of blackberry tea tannin oxidation

The results show that the acetone extract of the blackberry leaf has the ability to oxidize the total drug tea tannin. Said a little contrary to published data that the tea tannins are oxidized only by their own enzymes and show a clear inhibitory effect on enzymes other plants [17-18, 32]. However, these enzymes have been used in certain plants (barley, almonds, horseradish). Apparently in this regard blackberry leaves is advantageous exception.

In order to establish the presence of the enzyme preparation in the blackberry leaf O difenoloksidazy, experiments were performed in the presence of an inhibitor diethyldithiocarbamate. For this purpose 15 mg of sodium diethyldithiocarbamate dissolved in 2 ml of water. Count the activity carried on by polarograph ampermetrical. Reaction medium: 0.8 mL of citrate-phosphate buffer pH 5, 7, 0.2 ml of an aqueous solution of sodium diethyl dithiocarbamate, 10 mg drug acetone tea tannin. To this reaction mixture was added 0.35 M aqueous solution of tea tannin (312 mg in 2 ml water).

In the second experiment the reaction medium prepared by the above-noted sequences, was added 20 micro l aqueous solution phenolics blackberry leaf.

In both the first and the second case, there was no oxygen uptake on the basis of which it follows that the oxidation products of tea tannin and phenolic substances blackberry leaf caused by O-diphenol ocsidase. The results indicate an active part blackberry leaf enzymes in biochemical oxidation processes in the production of tea product.

Antioxidant Activity

The Ferric Reducing Ability of Plasma (FRAP) assay was used to measure the concentration of total anioxidants. UV/V is spectrophotometer M501 (Camspec Ltd, UK) was used for measurements of absorption changes that appear when the TPTZ-Fe3+ complex reduces to the TPTZ-Fe2+ form in the presence of antioxidants. An intense blue colour with absorption maximum at 593 nm develops. Standard solutions of 5.7 mM ascorbic acid in deionised water were prepared. Diluted standards or diluted extract samples were used on the day of preparation except the ascorbic acid solutions, which were used within 1h of preparation. An aqueous solution of 1000 µmol/L $FeSO_4x7H_2O$ was used for calibration of the instrument.

To measure FRAP value, 300 ml of freshly prepared FRAP reagent was warmed to 37 ^{0}C and a reagent blank reading is taken at 593 nm; then 10 ml of sample and 30 ml of water are added. Absorbance readings were taken after 0.5 s and every 15 s until 4 min. The change of absorbance ($\Delta A = A_{4min} - A_{0min}$) is calculated and related to ΔA of a Fe (II) standard solution. ΔA is linearly proportional to the concentration of antioxidant. One FRAP unit is arbitrarily defined as the reduction of 1 mol of Fe (III) to Fe (II). Antioxidant activity was expressed as an equivalent of ascorbic acid.

Table 4: The antioxidant activity of blackberry leaf and green tea

Sample	Antioxidant activity, mg/g of ascorbic acid equivalent
Experience	
Blackberry leaf fresh	1810
Blackberry leaf dried	10600
The dry extract of the blackberry leaf	11000
Control	
Fresh tea leaves	1015
Dried tea leaves	6000
Dry green tea extract	6500

From Table 4 shows that in terms of antioxidant activity of leaf extract of blackberry far exceeds commonly known green tea. This property blackberry leaf, particularly blackberry Caucasian, puts him in the ranks of the most powerful plant antioxidants.

Conclusions

- Investigated the chemical composition of the 6-leaf blackberry escape linking the growing season. It was found that the nature of the accumulation of volatile phenolic compounds: their minimum content to the same period of the beginning and end of the growing season (May and September).
- Established the character changes in the content of individual phenolic substances catechins, flavanols and leukoanthocyanidins blackberry leaf by month growing season.
- Examine the contents in the blackberry leaf of individual amino acids. It is shown that the identified 11 amino acids 5 are essential, making the blackberry leaf promising raw material for the production of tea products for children and dietary food.
- Were studied oxidative enzymes blackberry leaf and established the presence of the active form of
- O-diphenol oxidase.
- According to antioxidant activity much greater than the blackberry leaf green tea.

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