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BIOCHEMICAL CHARACTERISTICS OF CAUCASIAN BLACKBERRY LEAVES

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ABSTRACT. Some physical and chemical characteristics and 6-sheet blackberry shoot Caucasian (Rubus caucasicus L.) are studied. It was found that the minima of the moisture content, the extract substances and phenolic compounds are the same for the periods of the beginning and end of the growing season of plants. The composition of phenolic compounds are represented by catechins, flavonols and leucoanthocyanidins. The highest accumulation of phenolic compounds during the growing season is found in the middle of the season (July-August).

The average monthly amount of free amino acids blackberry leaf vegetation is 26.68 mg / g. 5 amino acids (His., Arg., Met., Leuc., Val.,) are mostly essential among the identified 11 individual amino acids. In the complex oxidative enzymes of blackberry leaf there have been revealed the presence of the active form of O-diphenoloxidase. Blackberry leaf and extract have high antioxidant activity.

KEY WORDS: amino acids; antioxidant activity; Caucasian blackberry; phenolic substances; physical and chemical composition; oxidative enzymes.

INTRODUCTION

Based on the analysis of data of plants used for the preparation of herbal teas and taking into account available resources and experience of their usage, we have chosen Caucasian blackberry leaves as the object of our study.

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. There are found 33 species in the Caucasus.

From these kinds of blackberries Caucasian blackberry (Rubus caucasicum L.) is mainly spread in Georgia.

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 is up to 300 thousand ha.

A distinctive feature of Caucasian blackberry brambles from other species is little prickly formative stems and leaves making easier the collection of raw materials.

What is essential is that the periods of vegetation of 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 for the primary processing of tea.

It should be noted that the information on the chemical composition of the plants are scarce, except of the works for the study of the texture of a 6-sheet escape blackberry and individual phenolic compounds flush elements [25,28].

The study of these questions determines the purpose of the study.

MAIN PART

MATERIALS AND METHODS

The objects of study were Caucasian blackberry leaves and the raw materials for the growing season such as: 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 wet combustion method [33], certain trace elements – by using a flame photometer [33], antioxidant activity - by the 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 end product quality. Phenolic compounds in the manufacture of tea leaf undergo a deep and diverse transformations that form the basis of tea production process. Obviously enormous work have been carried out by researchers to study the role and significance of tea phenolic compounds [1-6 12-13, 18-23, 36-49].

In this regard it's essential to study phenolic compounds used in the production of herbal tea or the vegetable raw materials.

Especially interesting is the establishment of seasonal dynamics of phenolic compounds during the growing season of the plant. Actually, the content of phenolic compounds in tea leaves is changeable during the processing season - for the Georgian tea plant the maximum of their savings in other similar conditions remains the same in 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 are given on the Table 1.

As a result, it was found that the nature of the accumulation of phenolic compounds in blackberry by growing season is identical to the tea leaves. In the Blackberry 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 leaves of blackberry leaves, as well as tea and other plant materials are fully in compliance with the basic laws of ontogeny of plants.

TABLE 1

Dynamics of phenolics blackberry escape during the growing season

##	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.	June	18,84±0,22	82,5
3.	July	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 our further work the first important issue is the study of the qualitative composition of phenolic compounds blackberry leaf.

Our previous work [26] established qualitative composition of phenolic compounds blackberry leaf consisting of three groups of flavonoids: leukoanthocyanidins, catechins and flavonols, which two-dimensional chromatograms are shown in Figure 1.

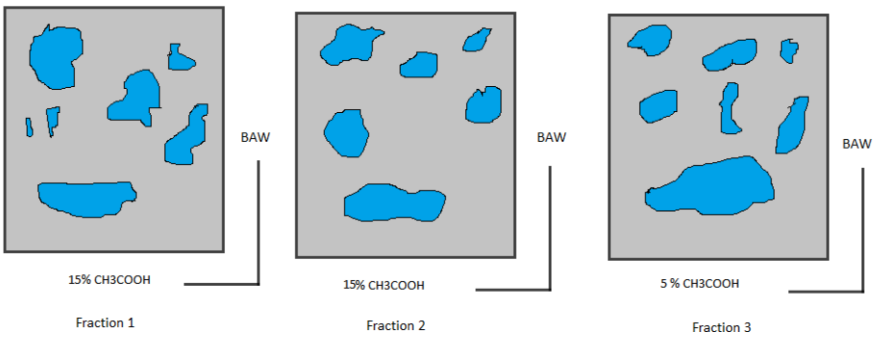

Fig.1. A two-dimensional chromatogram of a fraction of the total drug flavonoids of blackberry leaf

I- direction –BAW (n- butanol-acetic acid -water 4:1:5; the upper phase);
II –direction – a 15% acetic acid (lower phase)

The study of the dynamics of accumulation of flavonoid compounds in blackberry bine during the growing season revealed that after the flowering phase (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 bine and quantitative changes in vegetation indicate that these substances are actively involved in the metabolic processes of the plant cell [8, 11, 14, 16, 22].

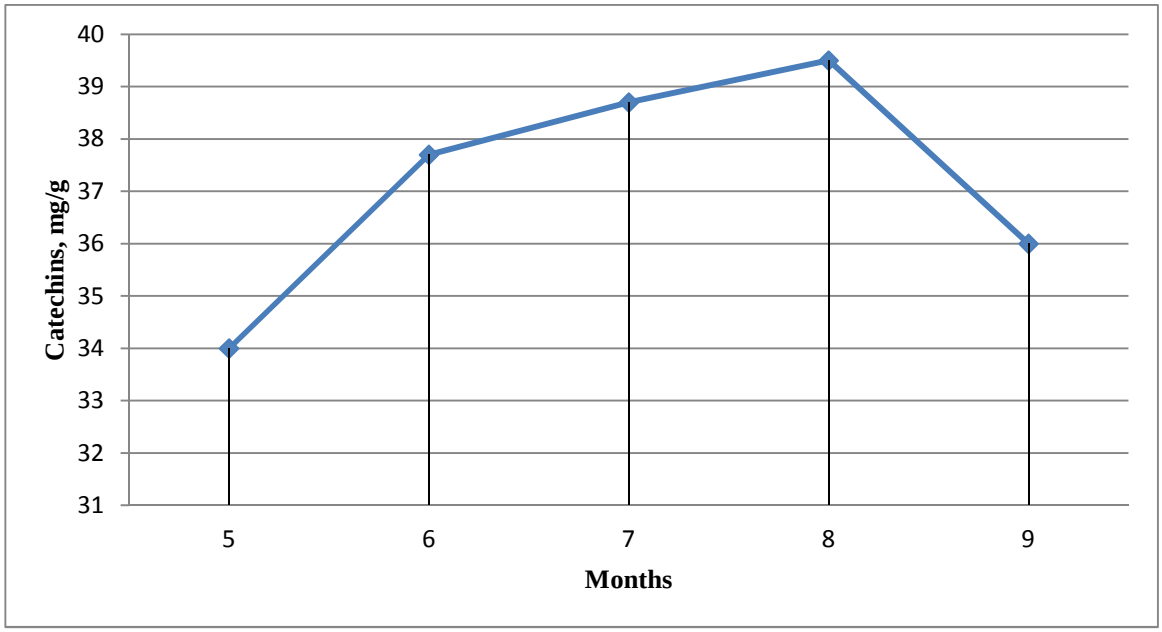

Fig.2. Dynamics of catechins blackberry bine during the growing season

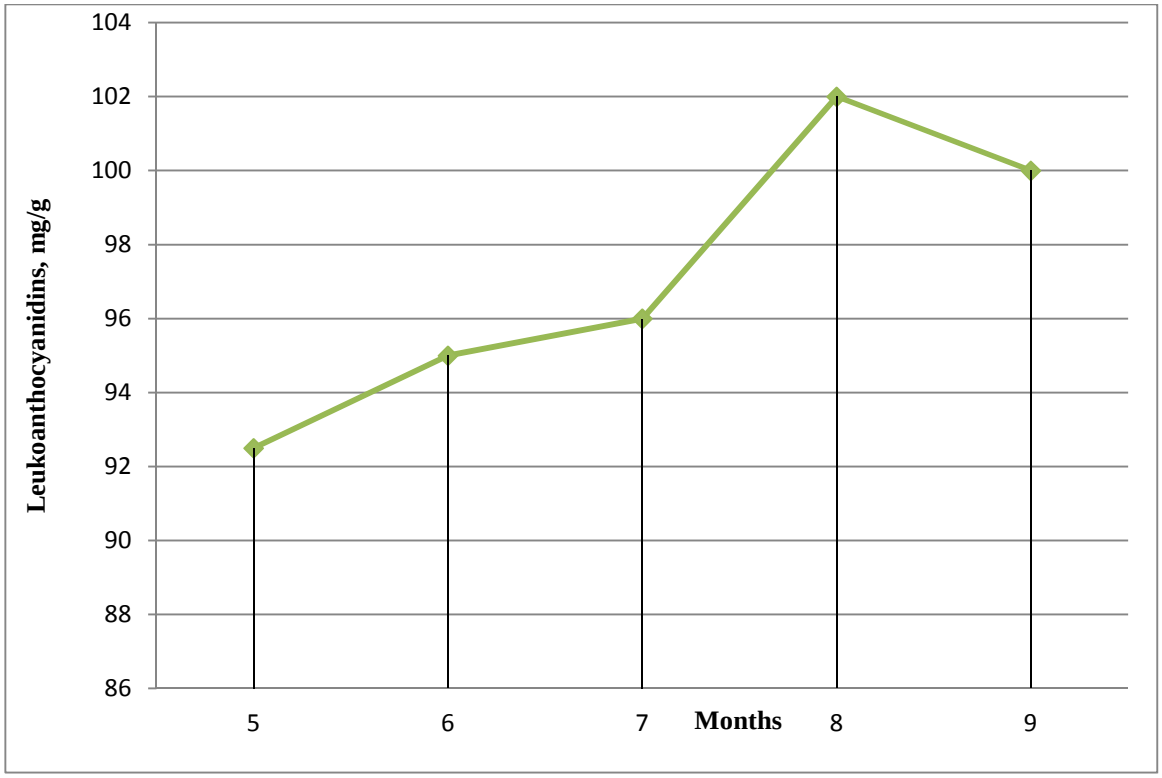

Fig.3. Dynamics of leukoanthocyanidins in blackberry bine during the growing season

It is known that many chemicals, including proteins and amino acids play an important role in tea producing. The basis of the structure of proteins is a complex combination of different amino acids.

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

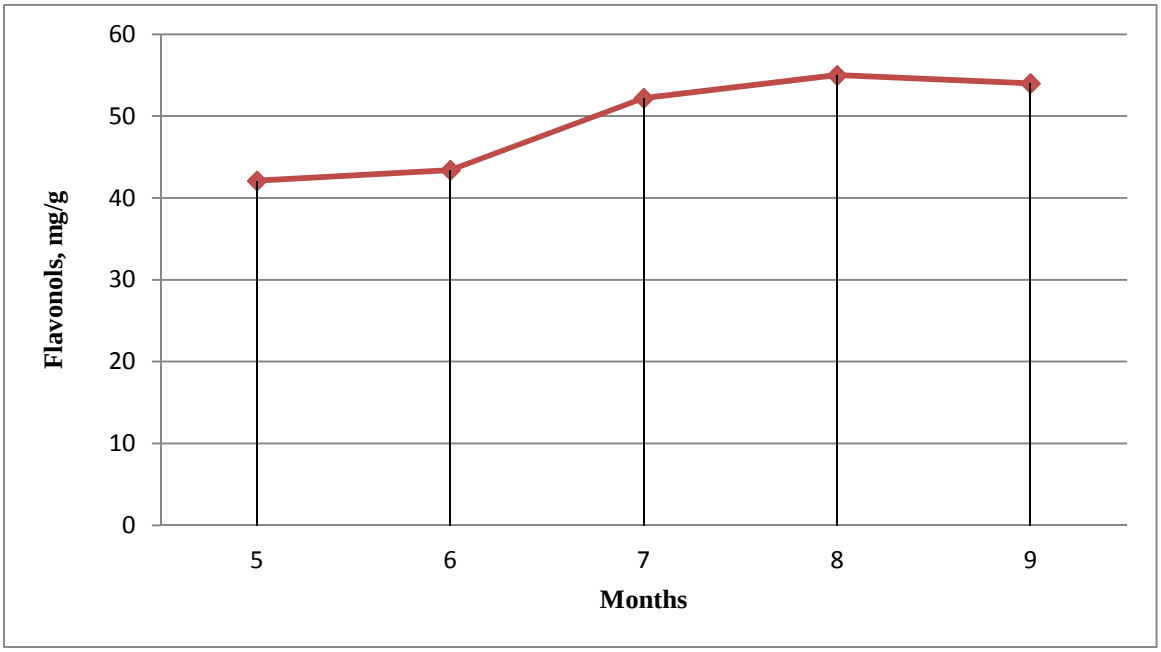

Fig.4. Dynamics of flavonols in blackberry bine during the growing season

Studies conducted by Soviet and foreign scholars related to the amino acid composition of tea leaves, are conducted for the 17 individual amino acids [7.41].

Given the importance of proteins and amino acids in the production of tea, we studied 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 a quantitative determination of the total amount of free amino acids in the context of the seasonal vegetation of 6-leaf blackberry shoot (Table 2) also has been carried out.

TABLE 2

The content of proteins and free amino acids in the blackberry bine during the growing season

##	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.	June	0,364	2,275	26,56
3.	July	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 is maximum, then gradually decreases and lows are in July-August. The maximum accumulation of free amino acids is represented in these months.

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 it can be seen, blackberry leaf is characterized by high-quality composition of individual amino acids: 5 amino acids (Histidine, Arginine, Methionine, Leucine, Valine) are mostly essential among the identified 11 individual amino acids. Comparison of quantitative content from tea leaf [10] indicates that the total content of lysine histidine + 7.8 times higher in blackberry leaf and leucine -9.1 times higher than in tea leaves (respectively 4.3 mg / g vs. 0 55 mg / g and 2.55 mg / g vs 0.28 mg g).

TABLE 3

The content of individual amino acids in the blackberry bine

##	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 of blackberry leaf tea is much superior (26.7 mg / g vs 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 the important amino acid histidine is essential for normal growth of infants [16] and take into account the lack of potent alkaloids, including caffeine, it provides effective basis for the widespread use of blackberry tea as a dietary and baby food.

Oxidative enzymes

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

In this regard we conducted a study of oxidative enzymes in the manufacturing of blackberry leaf based on the obtaining acetone extract of the blackberry leaf, and on the study of its activity against tea tannin and phenolic substances blackberry.

For the 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 leaf was treated in a homogenizer 2-3 times with a 1 minute cold (-15 0C) 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 ampermetric 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 ml 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 0C. Next calculated activity per 1 mg of acetone extract. It 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 was determined by ampermetric 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 0C.

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

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

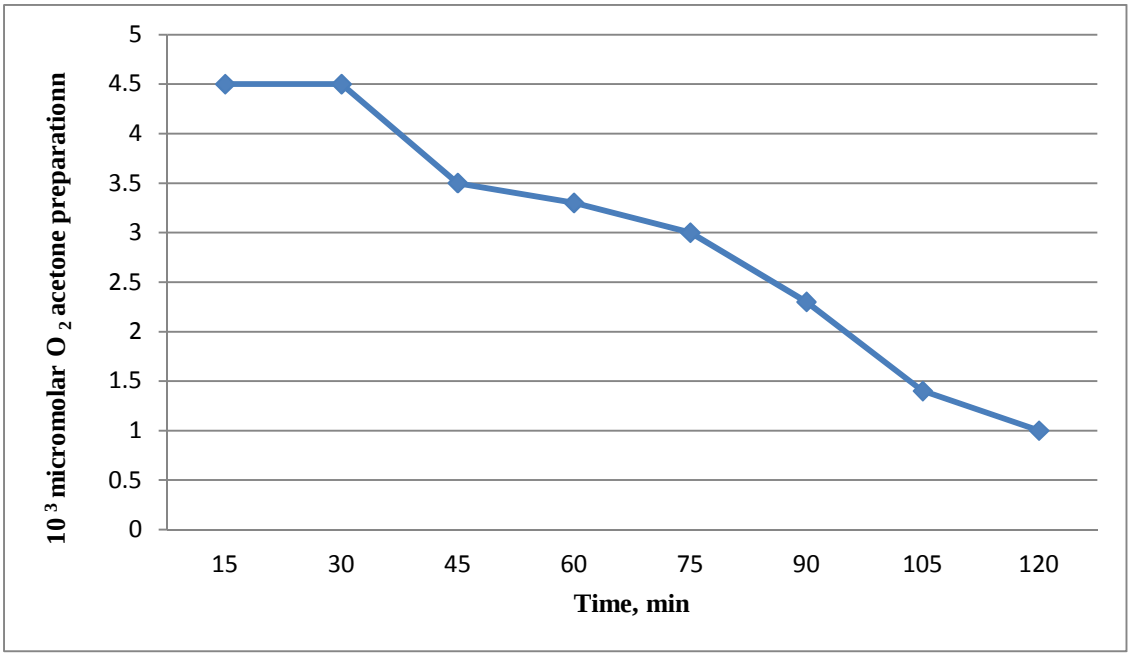

Fig.5. The activity of acetone extract of leaves of blackberry tea tannin oxidation

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

In order to establish the presence of the enzyme preparation in the blackberry leaf O diphenoloxidase, experiments were performed in the presence of an inhibitor diethyl dithiocarbamate. For this purpose 15 mg of sodium diethyl dithiocarbamate dissolved in 2 ml of water. Count the activity carried on by polarograph ampermetric. 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 cases, there was no oxygen uptake therefore the oxidation products of tea tannin and phenolic substances blackberry leaf caused by O-diphenoloxidase. The results indicate an active part of 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/Vis 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 FeSO4x7H2O was used for calibration of the instrument.

To measure FRAP value, 300 ml of freshly prepared FRAP reagent was warmed to 37 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 (ΔA = A4min - A0min) 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

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

CONCLUSION

- Investigated the chemical composition of the 6-leaf blackberry bine in the growing season. It was found 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 of changes in the content of individual phenolic substances catechins, flavanols and leukoanthocyanidins of blackberry leaf by month growing season.
- Examined the contents of individual amino acids in the blackberry leaf. It is shown that 5 amino acids are mostly essential among identified 11 amino acids, making the blackberry leaf as promising raw material for the production of tea products for children and dietary food.

● Were studied oxidative enzymes of blackberry leaf and established the presence of the active form of O-diphenoloxidase.

● Accordingly antioxidant activity of the blackberry leaf is much greater than it is of the green tea.

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კავკასიური მაყვლის ფოთლის ბიოქიმიური მახასიათებლები

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ანოტაცია. შესწავლილია კავკასიური მაყვლის (Rubus caucasicus L.) 6-ფოთლიანი დუყის ზოგიერთი ფიზიკურ-ქიმიური მახასიათებელი.

ნაჩვენებია, რომ მინიმალური ტენიანობა, ექსტრაქტული ნივთიერებებისა და ფენოლური ნაერთების შემცველობა აღინიშნება მცენარის ვეგეტაციის დასაწყისსა და ბოლოს. ფენოლური ნაერთებიდან მასში შედის კატეხინები, ფლავონოლები და ლეიკოანტოციანიდინები. მათი მაქსიმალური შემცველობა აღინიშნება სეზონის შუა პერიოდში (ივლის-აგვისტო). მაყვლის ფოთოლში თავისუფალი ამინომჟავების საშუალო შემცველობა არის 26,68 მგ/გ. იდენტიფიცირებული 11 ამინომჟავადან 5 შეუცვლელია (ჰისტიდინი, არგინინი, მეთიონინი, ლეიცინი, ვალინი). მაყვლის ფოთოლში აღმოჩენილია მაღალაქტიური ფორმის დამჟანგველი ფერმენტი O-დიფენოლოქსიდაზა. მაყვლის ფოთლებსა და ექსტრაქტს ახასიათებს დიდი ანტიოქსიდანტური აქტიურობა.

საკვანძო სიტყვები: ამინომჟავები; ანტიოქსიდანტური აქტიურობა; დამჟანგველი ფერმენტები; კავკასიური მაყვალი; ფენოლური ნივთიერებები; ფიზიკურ-ქიმიური შედგენილობა.

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БИОХИМИЧЕСКИЕ ХАРАКТЕРИСТИКИ ЛИСТЬЕВ ЕЖЕВИКИ КАВКАЗСКОЙ

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АННОТАЦИЯ. Изучены некоторые физико-химические характеристики 6–листных побегов ежевики кавказской (Rubus caucasicus L.). Установлено, что минимумы содержания влаги, экстрактивных веществ и фенольных соединений совпадают к периодам начала и конца вегетации растений. Состав фенольных соединений представлен катехинами, флавонолами и лейкоантоцианидинами. Максимумы накопления фенольных соединений приходятся в середине сезона вегетации (июль-август).

Количество свободных аминокислот листьев ежевики составляет в среднем 26,68 мг / г. Из идентифицированных 11 отдельных аминокислот 5 являются незаменимыми (гистидин, аргинин, метионин, лейцин, валин).В комплексе окислительных ферментов ежевичного листа установлено наличие активной формы О-дифенолоксидазы. Листья и экстракт ежевики обладают высокой антиоксидантной активностью.

КЛЮЧЕВЫЕ СЛОВА: антиоксидантная активность; аминокислоты; Кавказская ежевика; окислительные ферменты; фенольные вещества; физико-химический состав.