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The Leaf of Caucasian Rhododendron (*Rhododendron caucasicum Pall.)* for Receiving a Perspective Raw Material – "Mate" Type Tea

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Abstract

Diagnostic and dimensional characteristics of a Caucasian rhododendron leaves are investigated in this essay. Under the investigation are flavonoids substances of leaves and their transformation during vegetation process. It is derived that flavonoids substances contain the following components - catechins (19,2-36,1 mg / g), leukoanthocyanidins (164-209 mg / g) and flavonols (15,1-34,0 mg / g).

Character of change flavonoids changing is established like the following: in a flowering phase (May - June) their general content is slightly reduced (163,6 mg / g), their levels increas in August (279,1 mg / g) and decrease by the end of vegetation (211,8 mg / g); this work presents the new method of quantifying definition of arbutin by photoelectrocolorimetric method, which in comparison with existing (iodmetrical) method is characterized by expressed higher accuracy of definition (a degree of mistake of definition of 3,4 % against 4,8 % in comparison with an existing method).

Technological parameters of receiving tea product from the leaves of rhododendron are developed, providing preparation of raw material, cooling, crushing, roasting, thermal conditioning, repeated crushing and sifting.

It is shown, that the generated tea product differs both from black, and from green teas by organic characteristics and is more closely related to Paraguayan tea "Mate". This conclusion can serve as significant proof for expansion of a raw-material base of tea "Mate" in conditions of subtropics of Georgia.

Keywords: The Rhododendron Caucasian, Diagnostic attributes, Flavonoids, arbutin, Tea product "Mate"

1. Introduction

The rhododendron belongs to a family of Ericaceae D.C. Plants from this family are widely distributed in moderate climate areas of Northern hemisphere, especially in the mountains of Southeast Asia and in the Himalayas; a variety of rhododendrons grow up in the Arctic area, on Malay Archipelago, on New Guinea and in Northern Australia. In Georgia there are five varieties of the plant namely: a rhododendron Caucasian, Ponticum, Ungern, Smirnov and yellow (Good R., 1953; Flora of the USSR, 1952).

For practical use rhododendron Caucasian is by far the most likely candidate that belongs to endemic plants of Caucasus. The general area occupied by this plant in Georgia is roughly 120 sq.ga[Aleksandrova M.S, 1943].

Rhododendron is an evergreen bush that may reach 1,5 m in height; its leaves are dark leathery green and resemble an elongated oval, it is length 4,5-12 cm and sit s on a short, reddish stems; Plant's flowers ,which blossom in June – July, are light pink in color, gathered around the stem in 3 to 5 rows, and have pleasant aroma. The plant grows in a high-mountainous region at altitudes of 1600-3000 m above the sea level and forms extensive thickets in Alpine zone and a underbrush in subalpine woods. Rhododendron can be found in highlands of Georgia.

Depending of the habitat the leaves of a rhododendron Caucasian develop in May or June, twice in winter and at the end of the third year gradually wither away.

The structure of a leaf during the different periods of vegetation varies: the last year's leaves (semiannual with an impurity three-year) are prevailing in spring, and in the autumn - annual leaves can by found in larger quantities.

Based on the literary data leaves of a rhododendron contain terpens [Kurten S., 1971], acids [Oganesjan E.T., 1968], steroids [Grjaznova E.A., 1957], vitamins: C [Kezeli T.A., 1947] and P [Shalashvili A.G., 1973], phenols and their derivatives: arbutin [Zolotnitskaja S.J., 1958, 1965], catechins [Durmishidze S.V., 1960; Shalashvili A.G., 1967; 1970; 1973; Chrelashvili M.N., 1944], tannins [Grjaznova E.A., 1957; Zolotnitskaja S.J., 1958, 1965; Dzhaparidze L.I., 1945; Mejeninov M., 1929], flavonoids [Oganesjan E.T., 1968; Durmishidze S.V., 1960; Shalashvili A.G., 1970; 1973; 1967], anthocians [Shalashvili A.G., 1970; 1973; 1967].

Leaves of a rhododendron Caucasian are widely used in homeopathy at curing poisonings from mercury, diseases of mucous membranes and headaches [Aleksandrova M.S., 1975; Zemlinskij S.E., 1958]. Preparations from leaves possess high P-vitamin activity [Mejeninov M., 1929].

It is established, that both water, and alcohol-based methods of extraction from the leaves are effectual in fighting bacterial pathogenic microbes of intestinal flora, and against streptococci and purulent sticks [Gutnikova Z.I., 1947; Krylov G.V., 1969; Medvedeva R.G., 1952; Chervjakov D.K., 1953].

In folk medicine, the tincture prepared from plant leaves are use to treat gout, epilepsy, headaches, insomnia, intimate and diuretic conditions, rheumatism, dysentery, sharp and

chronic colic. Broth prepared from rhododendron is recommended by fork medicine as a cure against syndrome of oxygen insufficiency [Alekseev B.D., 1977].

Hydro-alcoholic tincture raises adaptation to physical pressure: it combines properties of a stimulator of muscular activity and a small tranquilizer [Karginova V.T., 1974].

Mountain population of Georgia use dried up leaves of a rhododendron Caucasian as effective tonic means and as a perfect substitute for tea [Kadaev G.N., 1963].

Analyzing above material, in our work we aimed to conduct a detailed study of characteristics of rhododendron leaves for the development of its practical adaptation.

2. Materials and Methods

The object of this research is Semi-annual leaves of a rhododendron Caucasian, collected from the high mountains of the Georgian Caucasus in the Alpine zones (Svanety and areas Racha-Lechkhumi) . A choice for semi-annually generated leaves was determined by preventing the intervention during natural ability of plant's life.

In a course of the study diagnostic attributes and dimensional characteristics of leaves, a complex of flavonoids substances,and the content of arbutin were examined.

Diagnostic attributes were determined via a microscopic method [The state pharmacopoeia of the USSR, 1987], dimensional characteristics - by measurement of a leaf.

The composition of phenolic compounds in Rhododendron leaves was studied in the preparation obtained by the following method. Flesh leaves (500 g) were ground in a laboratory mill and extracted with 80 % ethanol in a boiling water bath in a flask equipped with a backflow condenser. Extraction was performed for 40-60 min five times. The extracts were pooled, filtered through a glass filter no.2, and evaporated under vacuum at 50-60 ^{0}C to remove ethanol. The aqueous residue was repeatedly treated with chloroform to remove chlorophyll, resins, oils and other admixtures. Then, the extract was treated two or three times with ethyl acetate. Thus obtained extracts were pooled and evaporated under vacuum to obtain an amorphous mass [Durmishidze S.V., 1955; Durmishidze S.V, 1953]

To study the qualitative composition of phenolic compounds, the total preparation of phenolic compounds was fractionated on a column packed with a polyamide adsorbent. For this purpose, the preparation (15 g) was dissolved in distilled water and mixed with the polyamide adsorbent to obtain a thick mass. The mixture was loaded on a column (115 x 4 cm) packed with the polyamide adsorbent in the ratio 1:20. Elution was performed first by water and then with increasing concentrations of ethanol. The volume of collected fractions was 30 ml. The elution of catechins and leukoanthocyanidins was monitored by using the vanilla reagent, flavonols, under UV light and with the use of $AlCl_3$. Eluates were analyzed by two-dimensional paper (type C)[Durmishidze S.V., 1953; Zaprometow M.S., 1958].

In the first direction, chromatography was performed in the system of solvents consisting of butanol, acetic acid, and water (4:1:5); in the second direction, in 15 % acetic acid supplemented with the following developers: vanilla reagent (1 % vanillin in concentrated

HCl), 1 % solution of anhydrous $AlCl_3$ in ethanol, and 3% solution of p-toluene sulfonic acid in ethanol.

Three fractions were obtained. Fractions 1,2 and 3 were eluted from the column with 50, 60 and 90% ethanol, respectively. The qualitative composition of these fractions was determined by reactions with the vanilla reagent, p-toluene sulfonic acid, $AlCl_3$, as well as spectrophotometrically under UV ligh using an SF-4A spectrophotometer (Russia).

The quantitative composition of the preparation was studied densitometrically [Soboleva G.A., 1959]. For this purpose, chromatograms were treated with 1% silver nitrate in 25% ammonia, dried in the dark, and passed through the Shipalov densitometer [Shipalov M.S., 1962].

These experiments were performed by using a blue light filter with a maximum light transmission at 450 nm. The area under the absorption curve was measured with a planimeter and compared with the standard curves [Dzhemuchadze K.M., 1966].

For identification of arbutin two methods were used: standard [The state pharmacopoeia of the USSR, 1987] and the photoelectrocolo-rimetricall_developed by us [Melkadze R.G., 2009].

The essence of a new method consists of the following:

Analytical test leaves of a rhododendron in weight of 10 g were_crushed until the size of the pieces could go through a sieve with diameter of an aperture of 1 mm. About 0,5 g (weight exact) of crushed leaves were carefully crushed raw material was placed in a flask with 100 ml capacity , filled with 50 ml of water and boiled for 30 minutes on a water bath. Hot extraction was filtered through cotton wool in a measured flask with 100 ml capacity . Cotton wool with raw material were placed again in a flask, added 25 ml of water, washing off particles of raw material prior to that with a funnel into a flask and repeated the extraction process according to the method described above. Then the contents of a flask were filtered through a cotton wool in the same measured flask. The raw material on a left on a filtrate was washed out twice with 10 ml of hot water.

To a filtrate in a measured flask 6 ml of the sated solution of lead of acetate were added to the base solution, mixed and brought up with water to a label. After that, a flask was placed on a boiling water bath and maintained until full coagulation of a deposit was formed. A deposit was filtered through the folded filter. Surplus of lead of acetate of the basic was removed by adding 0,8 g a sulphicile sodium solution. The received extraction was filtered through the folded filter in a dry flask, forming thus the first fraction of a filtrate.

Capacity of 10 ml brought 4 ml of 0,02 % of a solution of sodium of nitrite in a measured flask and 4 ml of 0,08 % of a solution sulphicile_sodium. After 3 minutes in a flask were added 1 ml of a filtrate, 0,08 ml of 10 % of a solution of sodium hydroxide and lead up volume to a label. A solution was placed for 1 minute in the water bath which has been heated up to temperature 40-50 C and maintained at a room temperature for 20 minutes.

The optical density of the received solution was measured on photoelectrocolorimeter in a ditch with a 10 mm layer thickness, at length of a wave about 490 nanometers. Water was used as a comparison solution.

The contents of arbutin_in recalculation on absolutely dry raw material in percentage (X) calculated under the formula:

$$X = \frac{D \times 0{,}938 \times 10 \times 100 \times 100}{E^{1\%} \times m \times a \times (100-W)} = \frac{D \times 846{,}95}{m \times (100 - W)}$$

Where: D- optical density of a researched solution;

0,938 - factor of recalculation on waterless of arbutin;

$E^{1\%-}$ a specific parameter of absorption of arbutin at 490 nanometers;

m - weight of raw material, g;

a - the volume of extraction taken for the analysis, ml;

W - moisture content in raw material, %.

In addition, we have carried out an additional laboratory experiment on manufacturing tea product from the leaves of rhododendron_Caucasian following technological operations described below:

Fresh leaves were set up in Kokh's device with water ferry for 3-5 minutes, attached leaves were placed in a layer of 2-3 cm and cooled up to a room temperature then passed through a meat grinder such as "Kutter" and crushed until the size of particles reached 3-5 mm size;

The crushed raw material was then fried on laboratory skillet at a temperature of 150-160 ^{0}C 10-15 minutes, then cooled and placed in a laboratory dryer at temperature of 70-75 ^{0}C maintained for 4-6 hours. After that, the crushed raw particles were cooled and repeatedly crushed and sorted on vibrating sifters.

The received product was studied with taste approbation.

3. Results and Discussion

Based on the carried out microscopic analyses of leaves of a rhododendron Caucasian the following diagnostic attributes are established:

Cells of top epidermis polygonal are large, irregular-shaped and have highly skewed walls; lips are_numerous, large, rounded in share, are surrounded by two near by lips cells. There are numerous hair particles,, three cellular, thick-walled, resembling lace, are filled with light brown cytoplasm; the basic hair follicle is formed by two a pin outstanding of ring-shoped by the cells, located one above other (figure).

Dimensional characteristics of a a rhododendron Caucasian leaf structure is established on the average: length of a leaf of 13,16 cm, length of a stem 1,31 cm, width of a leaf of 6,06 cm (table 1).

From components of flavonoids substances (catechins, leukoanthocyanidins, flavonols) the basic are leukoanthocyanidins (approximately 75 % from a total sum of flavonoids substances) (table 2).

Character of change making of flavonoids substances of two year leaves of a rhododendron Caucasian during vegetation of a plant shows, that in a phase of flowering their content is slightly reduced, then grows in August and again decreases by the end of vegetation (table 3). Such way of a metabolism of flavonoids substances is similar to a metabolism of the given connections of a tea plant in conditions of subtropics of Georgia.

From the comparative data of quantitative definition of arbutin two methods were suggested by us first photoelectrocolorimetric method is characterized by higher accuracy and second with expressed (table 3).

A tea product derived from leaves of a rhododendron Caucasian on organoleptical parameters differs as from black, and green tea and it is closer approached to characteristics of a Paraguayan tea "Mate" that there can be rather significant for expansion of a raw-material base of tea "Mate" in conditions of subtropics of Georgia (table 4).

References

Aleksandrova M.S. (1975). *A rhododendron of natural flora of the USSR.* Publishing house "Science ", Moscow (in Russian).

Alekseev B.D. (1977). *Vegetative resources of Dagestan*. Makhachkala. (in Russian).

Chervjakov D.K. (1953). *About a diuretical action of rhododendrons*. Ulan-Ude. (in Russian).

Chrelashvili M.N. (1944). The contents soluble glucids in young and advanced age leaves of a rhododendron Caucasian. Messages AN of Georgia, 6,1,51, Tbilisi(in Russian).

Dmitriev A., Manjavidze L. (1961). *Rhododendron of Smirnov in Adjaria*. Messages AN GSSR, 30, 4,461(in Russian)

Durmishidze S.V. (1955.) Tannins and Anthocianins of Grapevine and Vine. Moskow, Akad. Nauk SSSR (in Russian).

Durmishidze S.V. and Nutsubidze N. (1953). *Trudy inst. Vinograd. Vinodel*. AN SSSR, 8,360 (in Russian).

Durmishidze S.V., Shalashvili A.G., Ushakova M.P. (1960). New sources bioflavonoids. Messages AN GSSR, Tbilisi, 25,6,673-676(in Russian).

Dzhaparidze L.I., Chrelashvili M.N. (1945). *A rhododendron Caucasian as a tannic plant*. Message AN GSSR, 6,2,139-142(in Russian).

Dzhaparidze L.I., Chrelashvili M.N. (1945). Losses tanids at preparation of rhododendron a leaves. Message AN GSSR, 6, 3, 214-216(in Russian).

Dzhemuchadze K.M., Nestyuk M.N. and Mileshko L.F. (1966). *Biochemistry and progressive Technology of Tea Production*. Moscow:Nauka, 110-115 (in Russian)

Flora of Georgia. (1952). Publishing house AN of Georgia, 7,5.(in Russian)

Flora of the USSR (1952). Publishing house AN of the USSR, Moscow, 18, 31.(in Russian)

Good R. (1953.) The geography of the flowering plants. New York

Grjaznova E.A., Podkolzina L.A. (1957). Materials to chemical studying a rhododendron yellow and Caucasian. Pyatigorsk(in Russian).

Gulisashvili V.Z. (1943). *Tannic plants of Georgia*. Publishing house AN GSSR, Tbilisi, (in Russian).

Gutnikova Z.I. (1947) *.A honey carryds plants of Primorski Krai.* Vladivostok(in Russian).

Kadaev G.N. (1963). Herbs of Karachaevo - Circassia. Circesc (in Russian).

Karginova V.T. (1974). Influence of a rhododendron on physical serviceability. Krasnodar. (in Russian).

Kezeli T.A., Chrelashvili M.N. (1947). An ascorbic acid and catalaza in leaves of a rhododendron in connection with vertical ash value. Messages AN GSSR, 8,6,413-416(in Russian).

Krylov G.V. (1969). *Grasses of a life and their selectors*. Novosibirsk(in Russian).

Kurten S. et al. (1971.) Uber Inhalstoffe verschiedener Rhododendron - Arten und ihre Kreislaufwirkung. Arch. Pharm, BD 304, H10, 5, 753-762

Medvedeva R.G. (1952). The Siberian herb - a rhododendron golden. Chemist's business 3,29-32(in Russian).

Mejeninov M. (1929). Tanincontains plants North Caucasus and an opportunity of use of their tanning industry. Moscow(in Russian).

Melkadze R.G. (2009). Arbutin leaves of a rhododendron Caucasian (Rhododendron caucasicum Pall), Barnaul(in Russian).

Oganesjan E.T., Bandjukova V.A., Shinkarenko A.L. (1968). Research triterpenoids and flavonoids Rhododendron caucasicum Pall. *Vegetative resources,* 4, 2, 240-243(in Russian)

Oganesjan E.T., Shinkarenko A.L. (1968). The quantitative contents tri-terpen acids in leaves of a rhododendron Caucasian, Dauricum and yellow. Pyatigorsk, (in Russian)

Shalashvili A.G. (1967). Flavonoids a rhododendron Caucasian-Rhododendron caucasicum Pall. Messages AN GSSR, 46,1,115-121(in Russian).

Shalashvili A.G. (1967). Reception of total preparations flavonoids from leaves Shkeri (Rhododendron ponticum L.) and a Deka (Rhododendron caucasicum Pall). Messages AN GSSR, 48,3,603, Tbilisi(in Russian).

Shalashvili A.G. (1970). Catechins,leukoanthocyanidins and flavonols a rhododendron Caucasian and Ponticum and their changes at vegetation. Tbilisi(in Russian).

Shalashvili A.G. (1973). Research flavonoids substances of leaves of a rhododendron Caucasian (Rhododendron caucasicum Pall), Tbilisi(in Russian).

Shalashvili A.G., Dzhishkariani O.M. (1973). The contents and quantitative change catechins, leukoanthocyanidins and flavonols in different bodies of a rhododendron Caucasian-Rhododendron caucasicum Pall. At vegetation. Alma-Ata(in Russian).

Shipalov M.S., Bokuchava M.A. and Soboleva G.A. (1962). *Proc. Inst. Plant Physiol.* USSR acad. Sci. Moscow: Nauka, 4,95 (in Russian).

Soboleva G.A. (1959). Biokhim. Chain. Proizv. no.7,176 (in Russian).

The state pharmacopoeia of the USSR (1987). The edition XI, Moscow(in Russian).

Zaprometow M.S. (1958). Fiziol. Ras't, 3.6-9 (in Russian).

Zemlinskij S.E. (1958). *Herbs of the USSR*, Moscow, 609. (in Russian).

Zolotnitskaja S.J. (1958, 1965). *Medicinal resources of flora of Armenia*. Yerevan(in Russian).

Table 1. Dimension of leaves of a rhododendron Caucasian

Number of measurement	Length, cm		Width of a leaves, cm
	A leaves	A stems	
1	14,7	0,8	6,1
2	15,1	1,4	5,8
3	13,8	2,0	5,5
4	14,2	1,9	4,8
5	10,2	0,6	5,4
6	13,0	1,0	6,0
7	12,8	0,8	5,4
8	12,6	1,6	5,2
9	14,4	2,0	5,8
10	10,8	1,0	5,0
Average	13,16	1,31	6,06

Table 2. The contents of flavonoids two years leaves of a rhododendron Caucasian (mg on 1 g air - dry weight)

#	Time of gathering	Catechins	Leukoanthocyanidins	Flavonols	Total
1	May	25,4	164,0	19,9	209,3
2	June	21,1	127,4	15,1	163,6
3	July	19,2	125,2	20,2	164,6
4	August	36,1	209,0	34,0	279,1
5	September	35,3	200,2	32,5	268,0
6	October	22,7	169,0	20,1	211,8

Table 3. The contents of arbutin in leaves of a rhododendron Caucasian (in % from air - dry weight)

Method	X	$\bar{x}$	S	P,%	$t_{(p,f)}$	ΔX	$\varepsilon_{,\%}$
Iodidemetrically (standarded)	1,87 2,03 1,79 1,94 1,81 1,96	1,90	0,093	95	2,57	0,0975	4,8
Photoelectrocolorimetrically (Suggested)	1,48 1,37 1,36 1,44 1,38 1,50	1,42	0,0602	95	2,57	0,063	3,4

Table 4. Organoleptical parameters of a tea product from leaves Rhododendron Caucasian

Parameters	Characteristics
Aroma	Feebly marked, "smoky"
Taste	Bitterish, pleasant, sated, specific
Insist	Chestnut-greenish
Tea leaves	With a green shade
Appearance	Grey - greenish from separate plates and stems

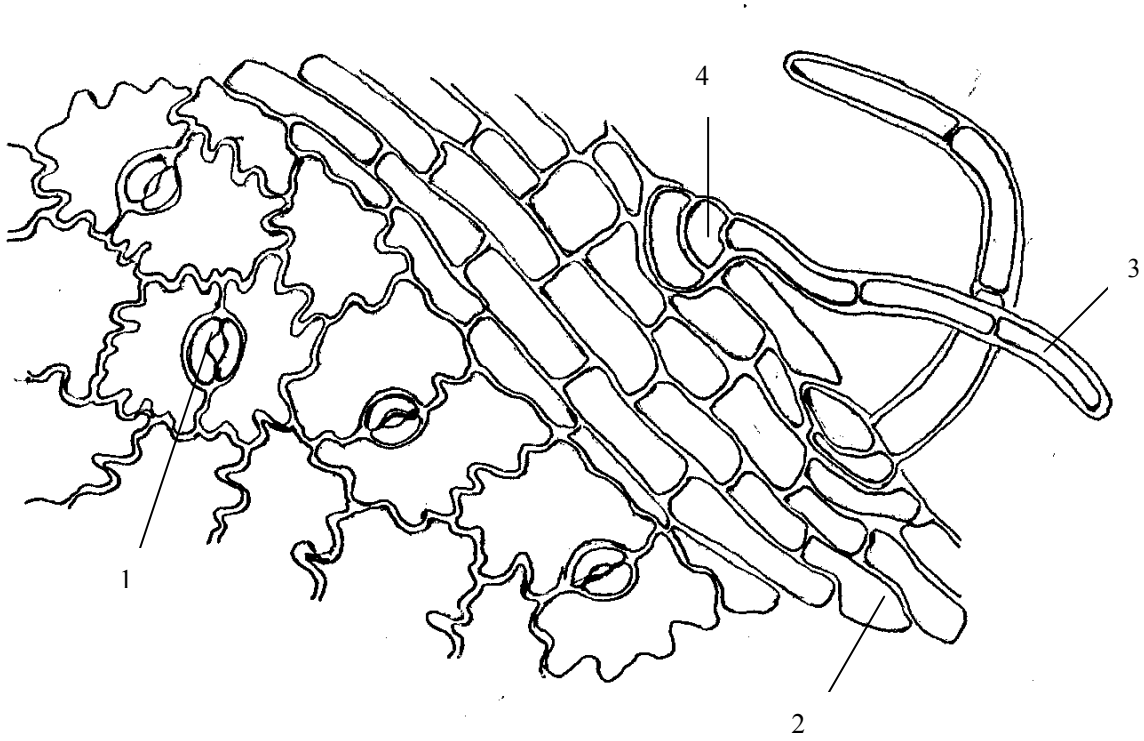

Figure. A structure of Caucasian rhododendron leaves epidermis

1-Paracitical type of a lip device

2- Twisting - wall of epidermis cells

3- Idle time ordinary hair

4- Ring cells on a place of hair attachment