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Plant Growing

Influence of Vertical Zoning on the Chemical Properties of Wild Growing Plants (*Prunus spinosa* L., *Crataegus pentagyna* Fing., *Crataegus pentagyna* W.et K., *Oxycoccos quadripetalus* Gilib., *Viburnum opulus* L.) in Georgia

Nino Gamkrelidze

Faculty of Agricultural Sciences and Biosystems Engineering, Georgian Technical University, Tbilisi, Georgia

(Presented by Academy Member Guram Aleksidze)

The polyphenolic composition of leaves and fruits of wild growing bushes in various ecosystems of Georgia were studied. The content of flavonoids, anthocyanins and antioxidant activity were determined. Influence of vertical zoning, abiotic and biotic factors on the chemical characteristics of wild growing plants were specified. The content of total phenolic compounds in the leaves of the samples collected in the highland region varies from 15.3-9.5 mg GAE/g in dry weigth (DW), the amount of flavonoids and monomeric anthocyanins is in the range 5.54-11.31 mg/g (DW) and 1.01-1.2 mg/g (DW). The content of total phenolic compounds in the fruit was 7.85-46.94 mg GAE / g in dry weigth, the amount of flavonoids and monomeric anthocyanins was 2.81-19.4 mg/g (DW) and 1.89-4.01 mg/g (DW), respectively. Indicators of similar chemical characteristics for lowland specimens are: total phenolic compounds for leaves 7.31-45.21 mg GAE/ g in dry weight, flavonoids 3.55-7.31 mg/g (DW), anthocyanins 0.85-1.8 mg/g (DW). Total phenolic compounds for fruit are 4.5-43.18 mg GAE/g in dry weight, flavonoids 1.61-9.23 mg/g (DW), anthocyanins 0.96-2.87 mg/g (DW). The obtained results clearly show the advantage of wild growing plants in the highland region in relation to raw materials rich in natural antioxidants and biologically active additive that allows us to use them in the production of food and pharmaceutical preparations. *© 2022 Bull. Georg. Natl. Acad. Sci.*

Polyphenols, flavonoids, anthocyanins, *Prunus spinosa* L., *Crataegus kyrtostyla* Fing., *Crataegus pentagyna* W.et K., *Oxycoccus quadripetalus* Gilib., *Viburnum opulus* L.

From antique time, people paid close attention to plants: studying, observing and searching for opportunities to use them in their own needs and requirements as vitaminized food, as means of treatment and prevention of various diseases.

Raw plant materials are rich in vitamins, trace elements, phenolic compounds and are characterized by high bioavailability. The growing interest in modern food production for raw plant materials is precisely related to phenolic

Table 1. Levels of total phenolic content, total flavonoids, total monomeric anthocyanins and antioxidant activity in leaves

Samples	TPC	TFC	TMA	DPPD	FRAP
*Prunus spinosa**	18.51±0.04	4.08±0.22	0.975±0.05	0.39±0.012	0.19±0.01
*Prunus spinosa***	44.76±0.08	11.31±0.9	1.2±0.005	0.65±0.025	0.28±0.07
*Crataegus kyrtostyla**	37.22±0.18	6.47±0.12	0.92±0.02	0.51±0.022	0.32±0.14
*Crataegus kyrtostyla***	48.98±0.1	8.86±0.17	1.01±0.003	0.63±0.014	0.46±0.06
*Crataegus pentagyna**	45.21±0.94	7.31±0.25	0.95±0.04	0.33±0.005	0.39±0.06
*Crataegus pentagyna***	49.5+0.27	9.69±0.19	1.05±0.05	0.71±0.003	0.54±0.16
*Oxycoccos quadripetalus**	14.2±0.017	4.11±0.03	0.85±0,001	0.33±0.015	0.21±0.02
*Oxycoccos quadripetalus***	19.56±0.008	8.23±0.01	1.1±0.05	0.69±0.06	0.49±0.01
*Viburnum opulus**	7.31±0.21	3.55±0.11	1.8±0.21	0.31±0.09	0.19±0.04
*Viburnum opulus***	15.3±0.03	5.54±0.02	1.15±0.04	0.71±0.05	0.55±0.07

*samples collected in Imereti; **samples collected in Mtskheta-Mtianeti.

Table 2. Levels of total phenolic content, total flavonoids, total monomeric anthocyanins and antioxidant activity in fruits

Samples	TPC	TFC	TMA	DPPD	FRAP
*Prunus spinosa**	20.82±0.12	9.23±0.28	1.27±0.003	0.32±0.9	0.11±0.018
*Prunus spinosa***	35.62±0.17	19.4±0.11	3.48±0.07	0.76±0.012	0.21±0.15
*Crataegus kyrtostyla**	17.62±0.12	6.12±0.19	2.3±0.005	0.35±0.03	0.29±0.11
*Crataegus kyrtostyla***	30.88±0.15	7.36±0.19	3.76±0.003	0.85±0.06	0.58±0.06
*Crataegus pentagyna**	43.18±0.24	8.88±0.14	2.87±0.04	0.59±0.01	0.39±0.08
*Crataegus pentagyna***	46.94+0.14	10.83+0.32	4.01±0.01	0.94±0.004	0.47±0.06
*Oxycoccos quadripetalus**	5.21±0.21	1.61±0.23	0.98±0.2	0.35±0.14	0.26±0.31
*Oxycoccos quadripetalus***	7.85±0.12	2.81±0.05	1.9±0.42	0.72±0.07	0.62±0.25
*Viburnum opulus**	4.5±0.25	1.9±0.056	0.96±0.08	0.41±0.05	0.27±0.06
*Viburnum opulus***	8.2±0.18	2.85±0.11	1.89±0.61	0.81±0.07	0.38±0.04

*samples collected in Imereti; **samples collected in Mtskheta-Mtianeti.

compounds. Phenolic compounds are produced practically in every cell and tissue of the plant [1-3]. They are non-food biologically active compounds and are characterized by antioxidant action, participate in the normal functioning of the enzyme system of a living organism, manipulate heavy metals and inhibit active forms of the elements [4]. Quantitative content of phenolic compounds in plants is related to taxonomic characteristics, vegetation period, geographical location and climatic conditions.

Materials and Methods

Chemicals. All chemicals used, including solvents, were of chromatographic grade.

Plant samples. Wild-growing plants blackthorn (*Prunus spinosa* L.), red hawthorn (*Crataegus kyrtostyla* Fing.), black hawthorn (*Crataegus pentagyna* W.et K.), small cranberry (*Oxycoccos quadripetalus* Gilib.) and viburnum (*Viburnum opulus* L.) ware collected from two different ecosystems in Imereti (550-600 m above sea level) and Mtskheta-Mtianeti (800-1100 m above sea level). Dry leaves and fruits of samples homogenized and were extracted by ultrasound using ethanol: water (0.75: 0.25 v/v) for 90 min at 42-45^{0}C [5]. The extracts were filtered.

Determination of total polyphenols. Quantitative analysis of phenolic compounds (TPC) was determined by the Folin-Chocalt method [6] on the spectrophotometer (FIBER OPTIC SPECTRO-

METER CECIL CE9500 Aquarius). Gallic acid was used as the comparable standard. The optical density of the extracts was determined at a wavelength of 760 nm (Tables 1,2).

Determination of total flavonoids. The total content of flavonoids (TFC) was determined by spectrophotometric method. The absorption was determined at a wavelength of 510 nm. The content of flavonoids is expressed in mg catechin equivalents per 1 g of dry raw material [7] (Tables 1,2).

Determination of total monomeric anthocyanins. Total monomeric anthocyanins (TMA) were determined by the pH-differential method [8]. Anthocyanins are characterized by reversible changes in molecular structure due to changes in pH.

As the molecular structure changes, so does the color of the anthocyanins. At pH 1.0, the color of the oxonium form predominates, and at pH 4.5, the colorless hemiketal form (Scheme) [8].

It is this reaction that underlies the pH-differential method, and allows the total amount of anthocyanins to be determined quickly and accurately, even when the mixture has other interferents compounds.

According to the dilution coefficient DC, two diluted solutions of the test samples are prepared – the first for them is a chloride buffer solution pH 1.0, and the second for them is an acetate buffer pH 4.5. The optical density of the samples is determined at λ_{max} 530 and 700 nm, respectively (Tables 1,2).

Optical density (A) of diluted samples is calculated by the formula:

$$A = \left(A_{530} - A_{700}\right)_{pH\,1,0} - \left(A_{530} - A_{700}\right)_{pH\,4,5}.$$

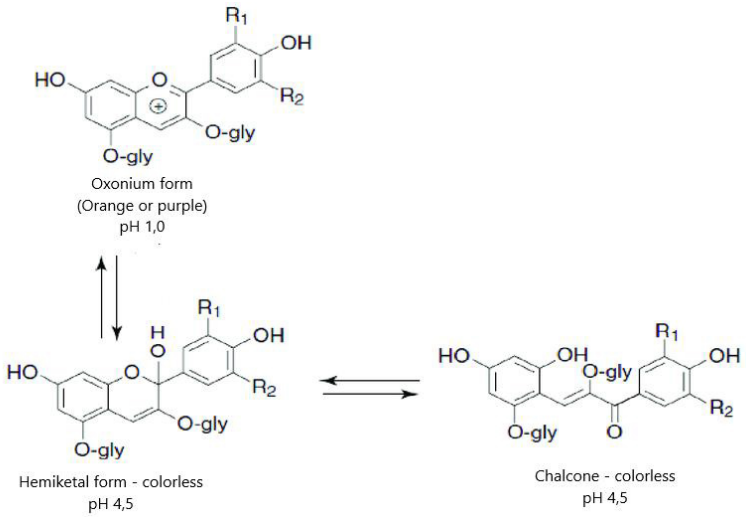
The concentration of monomeric anthocyanins in the initial sample is calculated by the formula:

$$MA = \frac{A \times Mr\left(C_{21}H_{21}O_{11}\right) \times DK}{\varepsilon}.$$

Determination of antioxidant activity by DPPH method. The antioxidant activity of the fruit and leaf extracts of the samples was studied by the DPPH method. The method is based on the property of antioxidants to bind stable chromo-radical (2,2-diphenyl-1-picrylhydrazyl) DPPH [9]. DPPH solution in methanol is dark purple in color, with the addition of solution containing antioxidants, the color intensity decreases to yellow, which is associated with the recovery of chromogen radicals. The optical density of the control and test mixture is determined at 515 nm (Tables 1,2).

The antioxidant activity of the test extract, inhibition coefficient (IC) is calculated:

$$IC = \frac{A_o - A_1}{A_o} \times 100$$



Scheme. Change in the molecular structure of anthocyanins.

Important properties of antioxidants are considered to be their ability to inhibit the catalytic action of metal ions during the oxidative processes of lipids. The restorative capacity of antioxidants can be determined by the so-called FRAP method [10]. The method is based on the ability of samples to reduce Fe^{3+} ions to Fe^{2+} ions.

The resulting complex is intense blue in color and is characterized by maximum absorption at 593 nm. A solution of ascorbic acid (1 mmol) is used for comparison. The reduction capacity of antioxidants is expressed in quantitative equivalents of ascorbic acid (Tables 1,2).

Conclusion

The article examines some of the wild plants of various species and ecosystems in Georgia. Analysis of the results shows that chemical characteristics are influenced by vertical zonality (height), abiotic and biotic factors. High levels of total polyphenols, total flavonoids, total monomeric anthocyanins, and antioxidant activity were observed in the leaves and fruits of samples collected in high mountain areas, compared with the same species growing in lowlands. Based on the results of the study, it can be assumed that relatively harsh climatic conditions in combination with genetic factors improve the formation of plant defense mechanisms.

The results clearly show the advantage of wild growing plants in the high mountain region in terms of raw materials rich in natural antioxidants and biologically active additives and allow them to be used in food and pharmaceutical production.

მემცენარეობა

ვერტიკალური ზონალობის გავლენა საქართველოში ველურად მოზარდი მცენარეების (*Prunus spinosa* L., *Crataegus pentagyna* Fing., *Crataegus pentagyna* W.et K., *Oxycoccos quadripetalus* Gilib., *Viburnum opulus* L.) ქიმიურ მახასიათებლებზე

ნ. გამყრელიძე

საქართველოს ტექნიკური უნივერსიტეტი, აგრარული მეცნიერებების და ბიოსისტემების ინჟინერინგის ფაკულტეტი, თბილისი, საქართველო

(წარმოდგენილია აკადემიის წევრის გ. ალექსიძის მიერ)

შესწავლილ იქნა საქართველოს სხვადასხვა ეკოსისტემის ველურად მოზარდი ბუჩქოვანი მცენარეების ფოთლებისა და ნაყოფის პოლიფენოლური შედგენილობა. განისაზღვრა ფლავანოიდების, ანთოციანინების შემცველობა და ანტიოქსიდანტური აქტივობა. დადგინდა ვერტიკალური ზონალობის, აბიოტური და ბიოტური ფაქტორების გავლენა ველურად მოზარდი მცენარეების ქიმიური მახასიათებლების შემცველობაზე. მაღალმთიან რეგიონში შეგროვებული ნიმუშების ფოთლებში საერთო ფენოლური ნაერთების შემცველობა მერყეობს 15,3–9,5 მგ, 1 გ მშრალ ნედლეულზე გადაანგარიშებით, ფლავანოიდების და მონომერული ანთოციანების რაოდენობა კი 5,54–11,31 მგ/გ და 1,01–1,2 მგ/გ ფარგლებშია. ნაყოფში საერთო ფენოლური ნაერთების შემცველობამ შეადგინა 7,85–46,94 მგ/გ მშრალ ნედლეულზე გადაანგარიშებით, ფლავანოიდების და მონომერული ანთოციანების რაოდენობა იყო 2,81–19,4 მგ/გ და 1,89–4,01 მგ/გ, შესაბამისად. დაბლობში მოზარდი ნიმუშებისათვის ანალოგიური ქიმიური მახასიათებლები – ფოთლებისათვის საერთო ფენოლური ნაერთები 7,31–45,21 მგ/გ, ფლავანოიდები 3,55–7,31 მგ/გ, ანთოციანები 0,85–1,8 მგ/გ, ხოლო ნაყოფისათვის საერთო ფენოლური ნაერთები 4,5–43,18 მგ/გ, ფლავანოიდები 1,61–9,23 მგ/გ, ანთოციანები 0,96–2,87 მგ/გ. მიღებული შედეგები ნათლად აჩვენებს მაღალმთიან რეგიონში ველურად მოზარდი მცენარეების უპირატესობას ბუნებრივი ანტიოქსიდანტებისა და ბიოლოგიურად აქტიური დანამატებით მდიდარი ნედლეულის თვალსაზრისით და იძლევა გამოყენების შესაძლებლობას სურსათის და ფარმაცევტული პრეპარატების წარმოებაში.

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