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Plant Physiology

Content of Antioxidants in Leaves of Some Plants of Tbilisi Environs

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ABSTRACT: Content of antioxidants - ascorbic acid, proline, anthocyanins, total phenols, proteins and soluble carbohydrates, also total antioxidant activity has been studied in leaves of 18 plant species, namely: *Scutellaria orientalis*, *Reseda lutea*, *Corylus iberica*, *Crataegus pontica*, *Crataegus kyrtostyla*, *Stachys lavandulifolia*, *Verbascum blattaria*, *Symphytum caucasicum*, *Melica uniflora*, *Juniperus polycarpos*, *J. excelsa*, *Nepeta supina*, *Lamium album*, *Anthyllis lachnophora*, *Primula saguramica*, *Trinia leiogona*, *Lathyrus roseus*, *Scrophularia divaricata*. Leaves of experimental plants were picked in surroundings of v. Kojori (East Georgia, environs of Tbilisi). According to experimental results leaves of the tested plants are rich of ascorbic acid, amino acid proline and total proteins. Soluble phenols and anthocyanins are in moderate amount. Some species revealed exclusively high antioxidant activity. All obtained indices may be taken into account while studying the medical properties of those tested species, which has not been used yet for this purpose. Moreover, experimental data may complete the data base about their chemical composition of the studied plants. *© 2013 Bull. Georg. Natl. Acad. Sci.*

***Key words**: ascorbic acid, proline, soluble phenols, anthocyanins, total proteins*

Isolation of active compounds from different plant material for nutritional, medical and cosmetic uses is very popular today [1]. Because of less popularity and non commercial use, many plants are not yet studied as the sources of antioxidants. Recent studies indicate that investigation of new plants and the knowledge about their ethnic and traditional use may offer many useful for human health improvement. Further chemical investigation of plants is necessary to reveal new plant sources of antioxidants, or to evaluate traditionally used products, and to create more perfect data base about their composition [2].

Recent determination of antioxidants and antioxidant system comprises a group of different class substances abating formation of active oxygen species on the one hand, and by neutralization of free radicals, formed as a result of spondaic oxidation of active oxygen, are responsible for detoxification and manifest protective effect upon biological structures [3-5].

Today plant antioxidants are considered as human protectors against progression of sclerosis and cardio-vascular diseases, diminishing the risk of cancer formation and declining ageing and increasing immunity, etc. [6]. Evidently revealing the antioxidant-rich plant source is very popular today. More the content of antioxidants in plant more is its practical value.

The Georgian flora counts about 4100 species. More than 400 species among them are used in folk and traditional medicine [7]; though, the antioxidant composition most of them is not fully investigated. Moreover, many species need further studies from medicinal point of view. Therefore, the complex investigation of representatives of Georgian flora for clearing the new plant sources of antioxidants, and evaluation of traditionally used products from this point of view, also creation of more perfect data base about the chemical composition of Georgian plants are necessary.

The purpose of the presented work was integrated investigation of the quantitative characteristics of antioxidants in plants of Tbilisi environs, comprising endemics of Georgia or Caucasus, and appreciation their total antioxidant activity.

Materials and Methods

Plant material was picked in surroundings of v. Kojori (East Georgia, environs of Tbilisi), in flowering phase (June-July). Content of antioxidants: ascorbic acid, proline, anthocyanins, total phenols, proteins and soluble carbohydrates, also total antioxidant activity has been studied in leaves of 18 experimental species, namely: *Scutellaria orientalis*, *Reseda lutea*, *Corylus iberica*, *Crataegus pontica*, *Crataegus kyrtostyla*, *Stachys lavandulifolia*, *Verbascum blattaria*, *Symphytum caucasicum, Melica uniflora*, *Juniperus polycarpos*, *J. excelsa*, *Nepeta supina*, *Lamium album*, *Anthyllis lachnophora*, *Primula saguramica*, *Trinia leiogona*, *Lathyrus roseus*, *Scrophularia divaricata* [8]. Tested plants belong to ornamental (*Melica, Lathyrus, Juniperus*), edible (*Corylus*, *Crataegus*), and medicinal (*Scutellaria*, *Corylus, Verbascum*, *Symphytum, Lamium, Scrophularia, Primula*) genera [9-11], but antioxidant content of most of them had not been investigated yet (*Reseda lutea, Nepeta supine, Anthyllis lachnophora, Trinia leiogona, Crataegus pontica, Primula saguramica, Verbascum blattaria, etc.*).

Content of ascorbic acid was determined by titration method with dichlorphenolindophenol. 2 g of leaves were ground in 15 ml of 2% hydrochloric acid and 10 ml of 2% metaphosphoric acid, and filtered. One ml of the filtrate was added to 25 ml of distilled water and titrated with a 0.001 M solution of dichlorphenolindophenol [12].

For proline investigation 0.5g of dry leaves were mashed with 10ml of 3% sulphosalicylic acid and 2 ml of filtrate added with 2 ml of acid ninhydrin and 2 ml of ice acetic acid. After 1h exposition on a water bath the extract was cooled and added with 4 ml of toluene and divided in a separating funnel. Optical density of upper layer was measured on a spectrophotometer (SPECOL 11, KARL ZEISS, Germany) at 520 nm [13].

While studying anthocyanins 200mg of dry leaves were placed in mix of 20 ml of 96% ethanol and 2 ml of 1% HCL for 24 h. Optical density of the obtained extract was measured at 540nm [12].

Soluble phenols were determined using Folin-Ciocalteu reagent. 0.5 g of leaf material was boiled in 80% ethanol for 15 min. After centrifugation residues of leaves were mashed in 60% ethanol and boiled for 10 min. Obtained extract was added to the first and evaporated. The sediment was dissolved in distilled water. One ml of the received solution was added with Folin-Ciocalteu reagent and optical density was measured at 765 nm. Chlorogenic acid served as control [14].

Content of proteins was studied after Lowry [15].

Soluble carbohydrates were tested by anthrone reagent [16]. 100mg of air-dry leaf material was added 96^{o} alcohol for extraction (3-fold extraction). Total amount of the obtained extract was evaporated on a water bath and dissolved in 5ml of distilled water.

Table 1. Content of ascorbic acid and proline in leaves of plants of Tbilisi environs

Plant species	Ascorbic acid, mg% (dry weigh)	Proline, μg/g (dry weigh)
Melica uniflora	43.38±1.3	84.0±3.4
Lathyrus roseus	48.2±1.8	279±13.4
Primula saguramica	12.5±5.1	29.0±1.3
Reseda lutea	69.89±2.7	354±13.8
Anthyllis lachnophora	72.3±2.8	204±6.1
Scrophularia divaricata	55.43±2.1	72±2.6
Verbascum blattaria	66.26±3.0	64±2.3
Corylus iberica	65.07±2.5	3.4±0.1
Symphytum caucasicum	51.81±1.7	268±8.6
Lamium album	72.3±.3.3	11.56±0.6
J. excelsa	55.43±1.9	58±2.4
Juniperus polycarpos	57.84±2.2	34±1.5
Scutelaria orientalis	101.22±4.0	89.4±3.7

0.5ml of the tested water extract was added 2ml of anthrone reagent and heated on a water bath for 10min. After this procedure test-tubes were placed in a cold water bath and 15min later optical density of the solution was measured at 620nm on a spectrophotometer (SPECOL 11, KARL ZEISS, Germany).

The total antioxidant activity of leaves was determined using modified DPPH (diphenylpicrylhydrazine) method [17]: 200mg of dry plant material was twice extracted with ethanol and extract was dried out. Obtained sediment was dissolved in 10ml of ethanol-water mix. 0.01ml of this extract was added with 40 μM solution of DPPH and after 30min of incubation in dark the optical density of the solution at 515nm was measured. The percent of inhibition was calculated.

Mean values of five biological replicates and their standard deviations are given in tables.

Results and Discussion

It is well known that ascorbic acid (vitamin C) plays an active role in many metabolic processes, like activation of vitamin B and folic acid, transformation of cholesterol into cholic acids and amino acid tryptophan – into neuromodulator serotonin. It is an antioxidant, which protects an organism against the free radical injury. It protects the immune system, diminishes the allergic reactions and supports the organism to fight infection [18].

Content of ascorbic acid in experimental plants varied in ranges of 32-120mg%. In particular, its highest amount was found in *Primula saguramika* (the endemic of Caucasus) and *Scutellaria orientalis* (Table 1).

Generally, if food contains more than 20% of the daily norm of ascorbic acid it is considered as vitamin C-rich product [19]. Accordingly, all tested plants may be regarded as rich of ascorbic acid, and this fact is an important positive moment while using them for medical purposes.

Proline takes part in collagen and cartilage formation. It retains muscles and joints flexible and diminishes skin flabbiness and shrinking caused by ultraviolet irradiation, or aging. Accordingly, proline additives may be useful in cases osteoarthritis, persistent soft tissue strains, and chronic back pain. The body makes proline from glutamic acid, and deficiency is rare in healthy individuals with a healthy diet. However, people recovering from traumatic injury, particularly skin injuries such as severe burns, may want to supplement this amino acid. People with pain caused by insufficient cartilage or collagen formation could benefit from extra proline in their diet as

Table 2. Content of soluble phenols and anthocyanins in leaves of plants of Tbilisi environs

Plant species	Soluble phenols, mg/g (dry weigh)	Anthocyanins, mg/g (dry weigh)
Melica uniflora	1.25±0.23	0.326±0.01
Lathyrus roseus	4.41±0.18	0.884±0.03
Primula saguramica	1.04±0.58	0.339±0.01
Reseda lutea	1.04±0.28	0.442±0.01
Anthyllis lachnophora	1.58±0.15	0.233±0.01
Scrophularia divaricata	15.17±0.38	1.468±0.04
Verbascum blattaria	1.75±0.71	0.518±0.01
Corylus iberica	2.83±0.24	1.588±0.07
Symphytum caucasicum	3.0±0.41	0.487±0.02
Lamium album	3.41±0.34	0.659±0.02
J. excelsa	14.17±0.47	0.255±0.03
Juniperus polycarpos	6.83±0.38	0.411±0.02
Scutelaria orientalis	4.83±0.17	0.164±0.02
Stachys lavandulifolia	16.67±1.41	1.292±0.05
Crataegus kyrtostyla	8.83±0.7	2.16±0.08
Crataegus pontica	7.33±0.51	1.858±0.08
Trinia leiogona	1.42±0.37	0.669±0.02
Nepeta supina	2.0±0.37	1.476±0.05

well. Proline may be in supplements used to promote cardiovascular health, usually in combination with vitamin C. The recommended therapeutic dose is between 500 to 1,000 milligrams daily, in combination with vitamin C [20].

Content of amino acid proline in most experimental plants was high. Especially must be mentioned: *Nepeta supina, Trinia liogona, Anthyllys lachnophora, Reseda lutea, Lathyrus roseus* (Table 1). Thus high content of proline also raises the pharmaceutical value of the studied species.

Widely spread in plant kingdom and abundant in our diet plant phenols are today among the most talked about classes of phytochemicals. Their positive role in some aspects of human health has been proved [21]. Anthocyanins are members of the ûavonoid group phytochemicals which are frequently referred to as bioûavonoids due to their multifaceted roles in human health maintenance. Anthocyanins in food are typically ingested as components of complex mixtures of ûavonoid components. Daily intake is estimated from 500 mg to 1 g, but can be several g/d if an individual is consuming ûavonoid supplements (grape seed extract, ginkgo biloba, or pycnogenol; The free-radical scavenging and antioxidant capacities of anthocyanin pigments are the most highly publicized. But, in fact, research clearly suggests that other mechanisms of action are also responsible for observed health beneûts [22].

Study of Soluble phenols and anthocyanins in experimental plants has revealed that in four species high content of phenols coincided with anthocyanins accumulation (*Crataegus pontica, Crataegus kyrtostyla, Scrophularia divaricata, Stachys lavandulifolia*) (Table 2). Tested plants may be divided in two groups by the content of phenols: spe-

Table 3. Content of total proteins and soluble carbohydrates, and total antioxidant activity in leaves of plants of Tbilisi environs

Plant species	Total proteins, %	Soluble carbohydrates, mg/g (dry weigh)	Total antioxidant activity, % of inhibition
Melica uniflora	3.2	1.2±0.03	24.2
Lathyrus roseus	3.84	1.2±0.03	42.5
Primula saguramica	3.0	0.6±0.01	32.9
Reseda lutea	3.52	1.4±0.05	28.6
Anthyllis lachnophora	22.40	1.1±0.04	39.9
Scrophularia divaricata	8.48	1.8±0.05	26.1
Verbascum blattaria	5.12	1.9±0.07	45.9
Corylus iberica	6.88	1.0±0.03	37.7
Symphytum caucasicum	4.0	1.1±0.04	27.5
Lamium album	3.36	2.3±0.06	54.3
J. excelsa	4.48	1.8±0.05	72.0
Juniperus polycarpos	4.88	2.3±0.07	77.0
Scutelaria orientalis	7.52	1.2±0.04	52.1
Stachys lavandulifolia	5.28	1.3±0.04	37.5
Crataegus kyrtostyla	3.40	2.1±0.06	775.0
Crataegus pontica	4.64	2.3±0.09	695.0
Trinia leiogona	1.50	0.5±0.01	41.2
Nepeta supina	4.52	1.0±0.03	53.1

cies containing phenols in relatively low amount (1-6mg/g), and in relatively high amount (6-17mg/g on dry weigh). Plants of the second group (*Crataegus pontica, Crataegus kyrtostyla, Scrophularia divaricata, Stachys lavandulifolia, juniperus isophyllos*) may be regarded as rich of phenols. High content of anthocyanins was determined in *Nepeta supina* and *Coryllus iberica*, but by the content of phenolic compounds these species were placed in the first group.

High content of soluble phenols and anthocyanins in the tested plants may be responsible for their medical properties, and taken into account while appreciating the pharmaceutical value of the studied species.

Proteins may serve as excellent antioxidant additives in food, because of their ability to inhibit lipid oxidation. Recently, bioactive peptides from enzymatic hydrolysis of various food proteins such as soy protein, casein, whey protein, gelatin, and wheat gluten have been shown to possess antioxidative activity [23]. Accordingly quantitative investigation of total proteins in experimental plants was interesting from this point of view.

Study of the content of total proteins in leaves of experimental plants revealed that *Scrophularia divaricata, Scutellaria orientalis* and *Corylus iberica* were distinguished by this index (Table **3**). Further investigations will show which protein in particular posses' antioxidative properties.

Soluble sugars seem to assume a dual role with respect to reactive oxygen species (ROS). They can be involved in ROS-producing metabolic pathways. In reverse, soluble sugars can also feed NADPH-producing metabolic pathways, such as the oxidative pentose-phosphate (OPP) pathway, which can contribute to ROS scavenging. Glucose has been shown to enhance cellular defenses against cytotoxicity of hydrogen peroxide in certain mammalian cell [24].

Lamium album, Juniperus polycarpos and *Crataegus* species were distinguished by relatively high content of soluble carbohydrates (Table 3) compared to other tested species. Presumably relatively low content of soluble carbohydrates in tested plant

will not have any negative effect, while using them as medication. Moreover, possibly the low level of soluble sugars is optimal for rising the antioxidative properties of plants, thus promoting the antioxidant efficiency of the studied herbs.

Crataegus species revealed exclusively high antioxidant activity among the investigated plant species (Table 3). According to the percent of inhibition juniper species possess high antioxidant activity as well. In most studied plants the percent of inhibition was lower than 50%, thus, their antioxidative activity was moderate.

According to experimental results it may be concluded that leaves of the tested plants are rich of ascorbic acid, amino acid proline and total proteins. Soluble phenols and anthocyanins are in moderate amount. Some species revealed exclusively high antioxidant activity. All obtained indices may be taken into account while studying the medical properties of those tested species, which has not been used yet for this purpose. Moreover, experimental data may complete the database about the chemical composition of the studied plants.

მცენარეთა ფიზიოლოგია

ანტიოქსიდანტების შემცველობა თბილისის მიდამოებში მოზარდი ზოგიერთი მცენარის ფოთლებში

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(წარმოდგენილია აკადემიის წევრის გ. ნახუცრიშვილის მიერ)

შესწავლილია ანტიოქსიდანტური ნაერთების – ასკორბინის მჟავას, პროლინის, ანთოციანების, ხსნადი ფენოლების, ჯამური ცილების, ხსნადი ნახშირწყლების რაოდენობა, აგრეთვე, ჯამური ანტიოქსიდანტური აქტივობა დაბა კოჯრის მიდამოებში (აღმოსავლეთ საქართველო, თბილისის შემოგარენი) მოზარდი 18 სახეობის მცენარეთა ფოთლებში – *Scutellaria orientalis*, *Reseda lutea*, *Corylus iberica*, *Crataegus pontica*, *Crataegus kyrtostyla*, *Stachys lavandulifolia*, *Verbascum blattaria*, *Symphytum caucasicum*, *Melica uniflora*, *Juniperus polycarpos, excelsa*, *Nepeta supina*, *Lamium album*, *Anthyllis lachnophora*, *Primula saguramica*, *Trinia leiogona*, *Lathyrus roseus*, *Scrophularia divaricata*. დადგენილია, რომ შესწავლილი სახეობების ფოთლები მდიდარია ასკორბინის მჟავათი და ამინომჟავა პროლინით. ასევე მაღალია მათში საერთო ცილების შემცველობა. ფოთლებში ზომიერი რაოდენობითაა ხსნადი ფენოლები და ანთოციანები. ზოგიერთ სახეობაში ძალიან მაღალია ჯამური ანტიოქსიდანტური აქტივობა. მიღებული შედეგები ყურადსაღებია სამედიცინო თვალსაზრისით ჯერ კიდევ შეუსწავლელი სახეობების გამოკვლევისას. ამასთან, მიღებული მონაცემები შეავსებს მონაცემთა ბაზას შესწავლილ სახეობათა ქიმიური შედგენილობის შესახებ.

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