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Dynamics of the Accumulation of the Biologically Active Substances Sanguisorba Officinalis L. Depending on Vertical Zonal

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Abstract*—The species *Sanguisorba officinalis* L. is widespread in the Republic of North Ossetia-Alania and is promising for the study and practical use of the pharmacological and food industry. The aim of the work is to analyze the dynamics of biologically active substances in the phytomass *S. officinalis* depending on the altitude gradient under conditions of RNO-Alania. Studies were carried out in 2012-2016. Samples of vegetable raw materials were taken at 5 stationary points located in various high-altitude belts: 1079, 1480, 1760, 1810 and 2300 m above the sea level. Studies were conducted using standard methods. The air-dry yield of *S. officinalis* above-ground phytomass in an averaged between 0.669 to 1.05 kg/m^2 over the years of research. The content of the studied biologically active substances in the above-ground phytomass of *S. officinalis* was found to change with increasing growth height. At a height of 1079 m, a high content of nitrogen-free extractive substances, hydroxycoric acids (in terms of chlorogenic), leukoantocyans, catechins, tanning substances was found in tissues. At a height of 1480 m, the accumulation of crude protein and routine was observed. At an altitude of 1810 m, fiber, ash elements, ascorbic acid, saponins (in terms of ursolic acid), and carotenes accumulate in grass. At a height of 2300 m, plant tissues show maximum levels of crude fat and flavonoids. The obtained data make it possible to estimate antioxidant and P-vitamin properties of *S. officinalis* and to recommend collection of vegetable raw materials of *S. officinalis* at different height to obtain certain biologically active substances. Effective and low-toxic natural polyphenolic antioxidants of *S. officinalis* can be used in the food, cosmetic and pharmaceutical industries.

Keywords—Sanguisorba officinalis L., biological resources Caucasus, biologically active substances, antioxidants

I. INTRODUCTION

The study of biological resources and physiologically active substances of wild medicinal plants is actively carried out all over the world. First of all, raw material and biologically active substances of plants included in official pharmacopoeia are studied, having a wide range of pharmacological activity for obtaining effective and safe medicinal and cosmetic phytopreparations [1]. They are studied as spice-flavouring additives and preservatives for food industry, etc. [2-4].

The Caucasus is one of the centers of the world biodiversity of plants due to the variety of natural conditions depending on the altitude above the sea level [5, 6].

In the mountain regions of the Republic of North Ossetia-Alania (RNO-Alania) the whole spectrum of vertical belts is presented: from high-mountain snowflakes and glaciers to dry heaths and semi-deserts. For successful development of plant wealth of the region it is important to carry out comparative study of peculiarities of growth, development of medicinal plants, accumulation of biologically active substances in ontogenesis in various ecotopes. A promising species for study and use in RNO-Alania is the widespread species *S. officinalis* L. – bloodlap medicinal. It is a perennial herbaceous polycarpic plant of the family *Rosaceae*. It contains a complex of biologically active substances (BAS): tannoglycosides, essential oils, tanning substances, flavonoids, saponins (sangvisorbine, lost), sitosterol, phenylcarboxylic acids (gallic, elagic, etc.), anthocyans, pectin substances [7-10].

S. officinalis is included into the State Register of Medicines of the Russian Federation. The structure of underground bodies (rhizomes and roots) is studied by *S. officinalis* which are raw materials for receiving the knitting, styptic, anti-inflammatory drugs [11-14].

Elevated bodies of *S. officinalis* are also considered as the raw materials. Compoundings of functional food stuffs with addition of flour from S. *officinalis* are developed. Flavonoids suggest to use as substitutes of synthetic preservatives in the meat-packing industry [15].

The biological resources of *S. officinalis* are studied in the Moscow and Oryol regions, in Bashkortostan, Khakassia and Tyva [16]. However works on studying of maintenance of BAS in *S. officinalis* raw materials in connection with high-rise zonality are conducted fragmentary. The relevance of these researches in RNO-Alania is obvious. It is necessary to establish how process of accumulation of BAS in *S.*

officinalis fabrics is connected with conditions of growth of individuals of a look.

The work purpose – the analysis of dynamics of maintenance of separate BAS in biomass of *S. officinalis* depending on a high-rise gradient in conditions RNO-Alania.

II. EXPERIMENTAL

Researches were conducted in 2012-2016 by a route method on A. I. Schröter [17]. During the work on the topographic map it was marked the key sites occupied by populations of each look, registered exact geographical coordinates of separate tsenopulyation, estimated the area and efficiency of thickets. Samples of wild-growing vegetable raw materials – a grass of *S. officinalis* – were collected in the territory RNO-Alania in 5 stationary points located in various high-rise belts from 1079 to 2300 m above the sea level:

1. Bottom of the left board of the valley of the Ardon River, Sadono-Unalsky hollow, northern outskirts of the village of Nuzal, slope of east exposure, 1079 m.

2. Right board of the valley of the Fiagdon, Nothern Jurassic depression, neighborhood of the village Mountain Dzuarikau. Rich in herbs and cereal meadow. Slope of the western exposure, 1480 m.

3. The basin of the Gizeldon, Nothern Jurassic depression, a northern spur of Bokovoy Range to the south of the Kakadursky pass. A rich in herbs and cereal meadow at the lower bound of extended thickets of a rhododendron yellow. Slope of northern exposure, 1760 m.

4. The basin of the Gizeldon River, a graded crest of a northern spur of Bokovoy Range to the south of Dargavskogo (Zelenogo). Slope of the west southwest exposure. A rich in herbs and cereal meadow with domination of a fescue of Voronov, 1810 m.

5. The basin of the Fiagdon River, a northern slope of Bokovoy Range is higher than Kora passage, an upper course of the Harisdon River. Cereal and rich in herbs subalpine meadow, 2300 m.

Laying of registration platforms was carried out according to the “Technique of definition of stocks of medicinal plants” providing the size of platforms from 0.25 to 4 sq.m [18]. The productivity of green material was estimated according to D. A. Muravyev [6]. Sampling was made according to the State Pharmacopoeia [19, 20]. Tests for a research stored in laboratory according to requirements [21].

Chemical analyses carried out in laboratories of Scientific Research Institute of biotechnology of FSBEI HE “Gorsky SAU” in 5 multiple frequencies. Determined by standard techniques: content of dry material, tannins [22]; a protein according to Kjeldal [23]; cellulose according to Gennfberg and Shtoman [24] crude ashes by method of dry combustion [25]; fat by Soksleta method [26]; the nitrogen-free extractive substances (NFES) – a calculation method; level of flavonoids, anthracene-derivatives – by techniques to the State pharmacopeia [19]; fenolglikozid according to N. I. Grinkevich [27]; ascorbic acid according to [28]; the general titrable acidity according to A. I. Ermakov [29]; carotinoids according to [30]; saponins according to A. V. Kiselyova et all. [11]. Indicators of maintenance of BAS were expected the absolute and dry mass of raw materials.

Statistical processing of results was carried out by means of the computer Microsoft Excel program with use of t-criterion of Student. Calculated r correlation coefficient: weak degree of interrelation – from 0 to 0.29, average – from 0.3 to 0.69, strong – from 0.7 to 1.0.

III. RESULTS AND DISCUSSION

Studying of a chemical composition of an elevated biomass of *S. officinalis* in various conditions of high-rise belts allows to create a better understanding about ecological and biological features and adaptive reactions of individuals of the studied type.

Productivity of the overground biomass of *S. officinalis* in an air-dry state on average for years of researches from 0.669 to 1.05 kg/m^2 fluctuated.

The value of a grass of *S. officinalis* depends on contents in it of a dry matter, the main nutrients (a protein, fats, NFES, ash constituents and also secondary metabolites (vitamins, organic acids, tannins, saponins, etc.) (Table I).

Content of a dry matter – an indicator which in many respects reflects orientation of processes of metabolism and adaptation of opportunities of plants to ecotope conditions.

Content of a dry matter in the land mass of *S. officinalis* on an average for 5 years depending on a high-rise belt changed in the range from 8.6% at the samples taken at the height of 1079 m up to 15.1% at the height of 2300 m. It have been observed a positive and statistically significant dependence of an indicator on height above sea level – r = 0.95-0.99.

Content of the ash constituents in dry matter of a grass S. *officinalis* depending on a high-rise belt changed from 5.8% (1079 m) to 7.6 – 6.9% at the height of 1810 and 2300 m respectively (r= 0.45-0.95).

An important component of the organic matter of plants are fats. In overground biomass of *S. officinalis* the content of fats is rather big and in average from 5.2% (1079 m) to 6.2% (2300 m) (r = 0.31-0.89).

The basic on weight, but the most hardly assimilable organic matter of plants – carbohydrate cellulose. Crude cellulose which is usually defined in biomass includes

TABLE I. NUTRIENT CONTENT IN THE ABOVE-GROUND MASS OF GARDEN BURNET ALONG THE ALTITUDE GRADIENT (2012 -2016)

Index	**Altitude, m above the sea level**				
	1079	***1480***	***1760***	***1810***	***2300***
Solid matter,%	8.6±1.2	9.7±1.1	11.5±1.3	10.7±1.4	15.2±1.0
Crude ash,%	5.8±0.04	6.5±0.05	6.3±0.03	7.6±0.05	6.9±0.06
Crude fat,%	5.2±0.06	5.8±0.07	5.7±0.06	5.9±0.07	6.1±0.06
Crude fiber,%	12.9±1.3	15.3±1.5	20.9±1.4	30.4±1.4	28.2±1.1
Crude protein,%	11.1±0.12	11.8±0.13	10.7±0.16	7.6±0.12	6.9±0.11
NFES,%	55.5±2.5	51.2±3.1	47.1±3.0	38.9±3.1	42.4±2.6

fraction of lignin. In process of increase in height the amount of crude cellulose in a grass of *S. officinalis* increased from 12.94% (1079 m) up to 30.48 – 28.23% at the height of 1810 and 2300 m respectively (r= 0.82-0.97).

The increase in fiber content usually leads to a decrease in the content of assimilable forms of carbohydrates (mono, oligo- and polysachars, primarily starch), as well as protein compounds. This pattern is also traced in our research.

As altitude rose above the sea level, the crude protein content of *S. officinalis* grass samples decreased from 11.2 - 11.8% (at 1076 m and 1480 m) to 6.9% at 2,300 m (r = -0.95 – -0.97). The crude protein content represents the total amount of nitrous substances. Nitrous substances are represented by proteins, free amino acids and amides, nucleic acids, nitrous bases and mineral forms of nitrogen. In the crude protein fraction, proteins typically accounts for 60-70% of total nitrogen and non-protein nitrogen compounds for 30-40%.

The main weight of carbohydrates of vegetative organs of herbaceous plants (except fiber) are easily assimilable substances, which are combined into a group of NFES. The content of NFES in *S. officinalis* tissues decreased from 55.5% (1079 m) to 38.9-42.4% at an elevation of 1810 and 2300 m, respectively (r = -0.87 – -0.92).

The total titrated acidity reflects the amount of hydrogen ions (pH) in the plant juice of *S. officinalis* grass, i.e. active acidity. Organic acids are usually weakly dissociated. For the index of total acidity there is a positive, statistically significant, dependence on altitude above the sea (r = 0.90-0.99) (Table II).

The value of *S. officinalis* is determined by the level of BAS content in its tissues. These are substances that are actively involved in the physiological regulation of the plant organism. They are responsible for plant immunity, resistance to a number of adverse environmental factors (drought, temperature changes, salinization or acidification of soil), improvement of seeds and fruits, their quality, etc.

TABLE II. THE CONTENT OF BAS IN THE ABOVE-GROUND MASS OF GARDEN BURNET ALONG THE ALTITUDE GRADIENT (2012 -2016)

Index	Altitude, m above the sea level				
	1079	***1480***	***1760***	***1810***	***2300***
Total acidity, %	2.6±0.04	3.0±1.1	2,8±0.1	3,6±0.1	3,7±0.1
Carotin, mg%	25,8 ±2.0	27,5 ±1.6	26,38±1.1	28,74±1.6	30.3±2.6
Ascorbic acid, mg%	151 ±11	169 ±10	151 ±9	180±6	175 ±8
Catechins, mg%	163±11	112±12	140±10	111±9	112±7
Flavonoids, mg %	21,4±2.1	28.7±1.1	17.1±0.7	20.4±1.1	21.7±0.5
Oxycoric acids, mg%	1.2±0.05	0.8±0.03	1.0±0.02	0.8±0.05	0.9±0.02
Leucoanthocyanins, mg%	613±21	415±18	438±17	458±19	365±23
Tanning material, %	7.4±0.2	6.0±0.4	6.3±0.3	7.0±0.4	7.4±0.5
Saponins, %	2.4±0.01	1.9±0.02	1.6±0.01	3.5±0.05	2.7±0.02

One of the most common in plant tissues is the carotene. The role of this pigment is great in the processes of photosynthesis, plant reproduction, and redox reactions. The pigment content of *S. officinalis* herb remained fairly stable at 25.5-30.6 mg%. There is a positive trend towards an increase in carotene content depending on the height belt.

The main active substances of *S. officinalis* are a variety of phenolic compounds. They participate in plant growth regulation by acting on processes through indolylacetic acid-indolylacetic acid oxidase system, have P-activity, are responsible for antioxidant activity, etc.

The level of ascorbic acid is also an important indicator of the antioxidant activity of *S. officinalis* tissues. In our experiments, the level of ascorbic acid in *S. officinalis* phytomass in averaged 165.84 mg% (r = 0.66, r = 0.90). This indicator also did not depend on the altitude above the sea level. A maximum amount of 180.32 mg% was contained at an altitude of 1810 m.

They are closely related to ascorbic acid in the metabolic processes of phenolic compounds: flavonoids, hydroxycoric acids, leukoantocyans and others. The content of bioflavonoids in plant raw materials is the most important indicator of its biological value. Flavonoid-containing plants are an important source of raw materials for the production of natural P-vitamin preparations having antioxidant properties. The wide spectrum of flavonoids activity is due to their versatile effect on numerous enzyme systems, functional activity of different cell systems.

The sum content of flavonoid compounds in *S. officinalis* tissues tended to increase as altitude increased (r = 0.64 – 0.90). Flavonoids are contained in plant tissues in a free state or as glycosides.

Catechins are flavonoids that are contained in tissues in a free state. They have a high physiological activity, easily oxidized. Their content in *S. officinalis* grass is in averaged 127.78 mg% over the years of research. The maximum content of 163.3 mg% is set at an altitude of 1079 m.

In the form of glycosides, plant tissues contain the flavonoid rutin. Its level in *S. officinalis* grass varied over the years of studies from 15.76 mg% in samples taken at 1760 m in 2013 to 30.05 mg% at 1,480 in 2012. On an average, the level of routine in *S. officinalis* grass was 21.86 mg%.

Depending on the altitude above sea level, the content of catechins and routine decreased (r = -0.50 – - 0.78 and r = -0.07 – -0.52 respectively).

The valuable chemical components of *S. officinalis* herb are also hydroxycoric acids (gallic, epigallocatechingallate, chlorogenic, coffee, ferulic), which, like flavonoids, are phenolic in nature. Their content (in terms of chlorogenic acid) in S. officinalis grass varied from 0.75 mg% (at 1810 m in 2014) to 1.29 mg% (at 1079 m in 2015). On an average, the content of hydroxycoric acids in *S. officinalis* herb was 0.92 mg%.

The negative relationship between altitude and content is established for leukoantocians (r = -0.60 – -0.85). Leukoantocyans are precursors of catechins and are directly related to the concentration of these compounds in plants [12].

Along with catechins, flavanoids are also the ancestors of condensed-row tanning substances. It is worth to remark that these condensed forms of compounds are capable of accumulation and higher content of leukoantocyans and catechins indicates low activity of tanning substances formation processes [12].

Tanning agents are a complex mixture of similar water-soluble phenolic compounds of high molecular weight capable of forming complexes with alkaloids, metals, gelatin and other proteins. Tanning substances have a high antioxidant activity. They interact with highly active free radicals arising from autoxidation, for example, lipid components, converting them into low-active ones [7, 31, 32].

The content of tanning substances in the above-ground mass of *S. officinalis* medicinal and on an average during the years of research was 6.84%. At an altitude of 1,076 m, the average tanning content over the years was 7.43%. However, there was no apparent trend and dependence of the tanning content on the sample collection site.

Among the secondary metabolite groups, saponins are of great interest. They are protective factors in plant relationships with other organisms and also serve as raw materials for the pharmaceutical industry. In the above-ground mass of *S. officinalis*, the content of saponins (in terms of ursolic acid) changed on an average over the years of research in the range of 1.66% at an altitude of 1,760 m to 3.56% at an altitude of 1,810 m. Dispersion analysis did not establish the effect of the season and altitude above sea level on the content of saponins. However, a strong influence of the interaction of factors was revealed.

Thus, as growth height increases in *S. officinalis* green mass, the dry matter, crude fiber, crude fat, crude ash, general acidity, ascorbic acid, saponins, carotene, flavonoids, and polysaccharides content increases.

The content of catechins, leukoanthocians, oxycoric acids (in terms of chlorogenic), on the contrary, decreases, which is probably related to the protective role and factor of adaptation of plants to mountain conditions of the environment (intensity of solar radiation, high temperature, etc.

The maximum content of catechins (163 mg%) and leukoanthocyans (612 mg%), hydroxycoric acids (1.2%) is determined in *S. officinalis* collected at the foot of the left side of the Ardon River Valley, Sadono-Unal pits, the northern edge of the village of Nuzal, the slope of the eastern exposition, 1079 m above the sea level.

IV. Conclusion

S. officinalis is a massive species in the republic, and has a wide range of distribution. The results provide a comprehensive assessment of the use of *S. officinalis* as raw materials.

The air-dry yield of *S. officinalis* above-ground phytomass averaged between 66.9 and 105.0 c/ha over the years of research. The content of the studied BAS in the above-ground phytomass of *S. officinalis* was found to change with increasing growth height.

At a height of 1079 m, a high content of nitrogen-free extractive substances (55.5%), hydroxycoric acids (in terms of chlorogenic) (1.29 mg%), leukoantocyans (612 mg%), catechins (163.3 mg%), tanning substances (7.43%) was found in tissues.

At a height of 1480 m, the accumulation of crude protein (11.8%) and routine (30.05 mg%) was observed.

At an altitude of 1810 m, fiber (30.48%), ash elements (7.6%), ascorbic acid (180.32 mg%), saponins (in terms of ursolic acid) – 3.56%, carotenes (31.0%) accumulate in *S. officinalis* grass.

At a height of 2300 m, plant tissues show maximum levels of crude fat (6.2%) and flavonoids (1.74%).

The obtained data make it possible to estimate antioxidant and P-vitamin properties of *S. officinalis* and to recommend collection of vegetable raw materials of *S. officinalis* at different height to obtain certain BAS.

Effective and low-toxic natural polyphenolic antioxidants of *S. officinalis* can be used in the food, cosmetic and pharmaceutical industries.

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