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Identification and Analysis of the Content of Biologically Active Substances of Juniper Cone Berries and Their Antioxidant Activity

Myroslava Hrytsyna [1,*], Ivan Salamon [2,*], Ruslan Peleno [1] and Vladimira Vargova [3]

[1] Faculty of Social Development and Health, Stepan Gzhytskyj National University of Veterinary Medicine and Biotechnologies of Lviv, 50, Pekarska St., UA-79010 Lviv, Ukraine; andriyovich30@ukr.net
[2] Department of Ecology, Faculty of Humanities and Natural Sciences, University of Presov, 01, 17th November St., SK-081 16 Presov, Slovakia
[3] Research Institute of Plant Production, Grassland Mountain and Agriculture Institute, 36, Mladeznicka St., SK-974 21 Banska Bystrica, Slovakia
* Correspondence: hrytsynamr@lvet.edu.ua (M.H.); ivan.salamon@unipo.sk (I.S.); Tel.: +380-97255-1798 (M.H.); +421-904150750 (I.S.)

Abstract: Juniper cones are widely used in the food industry and medicine. The aim of this study was to identify juniper growth sites with the highest concentrations of carbohydrates, fats, organic acids and phenols in their cone berries, as well as to assess their antioxidant properties. Research was conducted on 23 different populations from Slovakia, along with fruits purchased from commercial sources in Albania, Bulgaria, Bosnia, Slovenia, and the Czech Republic. The content of fermentable sugars in industrial samples was higher in *Juniperus communis* than in *J. oxycedrus* (360 and 197 g/kg, respectively), and 20% higher in samples from Albania than those from Slovakia. The carbohydrates included glucose and fructose; the latter was 35% less. We selected groups based on a cluster analysis of the content of organic substances in juniper cone berries and their antioxidant activity, collected from different Slovakian populations. So, based on the conducted research, it was established that the content of organic substances, particularly sugars, in cone berries decreased with elevation in the mountains and was the best at medium altitudes. At the same time, the antioxidant effect was better in cone berries collected on the north side at all altitudes. The best in terms of the content of phenols and antioxidant activity were the populations located on the northern, rather steep slopes in the lowlands of the Slovak Carpathians. These indicators fell with the increase in altitude in the mountains, especially from the southern side. The best places to collect raw materials are populations from middle latitudes: Chhrámec, Teplá dolina, lokalita 1, Horné lazy, Iliaš, Priechod-South, Priechod-West, Spišsky hrad, Selčianske sedlo, Cerovo, Poprad, Kišovce–Hôrka, Ostrá hora, Teplá dolina 2, Kráľová 2, and Kráľová 1. The cone berries can be used as the raw material base for producing the Slovak national alcoholic drink "Borovichka".

Keywords: antioxidant methods; juniper berry; organic compounds; biological activity

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1. Introduction

In recent years, interest in medicinal products of plant origin has increased. Searching among species for their natural populations with the highest content or best composition of biologically active substances is becoming an increasingly urgent issue today. Common juniper (*Juniperus communis* L.) is one of these species, which grows on stony, infertile soils, in fields, meadows, pastures, in open forest glades, and other places, almost from sea level to alpine places. These are shrubs of various shapes (columnar, oval, and bushy) up to 2.0 m high.

Juniperus communis L. belongs to the family *Cupressaceae*, order *Pinales (Coniferales)*, Class *Pinopsida (Coniferopsida)*, divisio *Pinophyta (Gymnosperma)*. The area of *J. communis* covers Europe, Asia, and North America, and it occurs in North Africa. In Slovakia, it grows in coniferous undergrowth, and less often in mixed forests in the lower mountain forest belt in the Carpathians. It is widely cultivated in European countries and is grown as an industrial crop.

J. communis is the most widespread conifer species in the world, with a circumpolar distribution extending from about 70° N latitude in Alaska, Scandinavia, and Siberia to approx. 28° N in the Himalayas. It is a pioneer forest species that occupies natural rock outcrops and other places with bare soil and intense sunlight, both in broad-leaved and coniferous forests (especially *Pinus sylvestris*—*Betula* spp.—*Quercus* spp. *associations*), in which it can have local dominance in places where log cabins are situated (without fire). It is common in open forest–grassland ecotones on poor sandy soils and stabilized inland dunes. It is often found together with species such as *Calluna vulgaris*, *Erica* spp., *Vaccinium* spp., *Arbutus* sp., *Cytisus scoparius*, *Ulex* sp., *Salix* spp., as well as in the mentioned tree canopies. It grows at altitudes from 5 m to 2400 m above sea level, is not selective about the type of soil, and is found on dry sand, chalk rocks, and loose (dolomite) scree, as well as in acidic peat with low or fluctuating groundwater levels. No range-wide threats have been identified for this species [1].

In Slovakia, *J. communis* is common in pine forests in the lower part of the Carpathians. The places of collection of raw materials for the investigation were located in different biotopes—from open to dense stands of the species together with other photophilous tree species, mainly shrubs, which are found in grass–herb or shrub plant groups. Common juniper most often spreads on extensive pastures, and thanks to its sharp pine needles, it is not threatened by grazing. On the contrary, it has a competitive advantage because the plants usually grow on rocky, infertile soils, in fields, meadows, pastures, open forests, and other places, from almost sea level to alpine places.

Juniperus communis is a bush or a low monoecious or dioecious evergreen tree with grayish-brown bark. Branches are hanging or almost hanging; twigs are obtuse triangular. The leaves are ring-shaped, three in each, lanceolate, prickly, grooved on the upper side, swollen callus at the base, and bluntly keel-shaped on the lower side. On top, conifers are whitish with a wax coating, shiny, green on the bottom, and jointed at the base. They are stored on the branches for four years [2].

Male and female cones are on axillary shortened branches covered with small, scaly leaves. Male sporulation organs have the appearance of yellow spikelets. Male cones are oblong and almost sessile; their scales have three to four anthers, broadly ovate, elongated, and pointed. Female cones are almost globular and consist of three to six crosswise or three scales each and three seed primordia, which, as they mature, become fleshy and grow to form a juicy green cone berry of a rounded shape (7–9 mm in diameter). Unripe fruits are often ovoid, two or three times shorter than a leaf. These are not berries but cones with fleshy scales that have grown together. After a year, the cone berries turn black with or without a wax coating, often with three bumps on the top. The seeds are oblong with a hard shell, compressed on top, with a point; on the sides, there is a depression with bubbly resinous glands [2].

Red juniper (*J. oxycedrus* L.) (*J. rufescens* Link) is a dioecious evergreen tree, rarely a shrub. Leaves on top have two white stripes separated by a central green vein, very keeled, up to 2 cm in length, and 1.3–2 mm wide. Cone berries are black-brown, 6–12 mm in diameter [2].

J. oxycedrus grows in the Mediterranean region and the Middle East. Common in Mediterranean sclerophyllous shrubs and dry deciduous forests, where it grows together with *Pinus*, *Carpinus betulus*, *Quercus*, and *Quercus-Lentiscus*, as well as in mountain and wet forests with *Cedrus libani*, *Pinus nigra*, *Juniperus foetidissima*, and *Juniperus excelsa*. Altitude range: 1–2200 m. It is found in dry, stony slopes on thin soils. It is mainly limited to regions with a Mediterranean climate but can grow in more continental conditions in the Balkans and the Iberian Peninsula. In Ukraine, it grows on dry, open, rocky slopes in the mountainous Crimea.

Common juniper is widely used as an ornamental plant in parks and gardens, especially creep varieties, and numerous varieties have been bred from it. In Ukraine, there is experience in the introduction of conifers, in particular, *Juniperus* in microlandscape dendrocompositions of various types of collection plantations of the Kryvyi Rih Botanical Garden. Their perspective for further use in greening industrial cities of the steppe zone of Ukraine has been set [3].

The *European Pharmacopoeia 8.0*, 2013 [4] includes Juniper—raw material Juniperi pseudo-Fructus, definition dried ripe cone berry of *Juniperus communis* L. with content: minimum 10 mL/kg of essential oil (anhydrous drug); intensely aromatic odor, primarily if crushed. Juniper oil (*Juniperi aetheroleum*). Definition: Essential oil is obtained by steam distillation from the ripe, non-fermented berry cones of *Juniperus communis* L. In the latest edition of the *European Pharmacopoeia* monograph, this plant is now described as Juniperi galbulus. According to the *European Pharmacopoeia* (Ph. Eur. 8.0, 2013), the following components of the essential oil have been identified: α-pinene (20–50%), sabinene (maximum 20%), β-pinene (1–12%), β-myrcene (1–35%), α-phellandrene (maximum 1.0%), limonene (2–12%), terpinen-4-ol (0.5–10%), bornyl acetate (maximum 2.0%), β-caryophyllene (maximum 7.0%).

The seeds and fruits of the plant contain d-α-pinene, camphene, pectins, glycolic acid, malic acid, formic acid, acetic acid, cyclohexitol, terpene, proteins, fermentable sugars, wax, gum, ascorbic acid, dihydrojunene, α-pinene, hydrocarbon junene, cadinene, juniper, and camphor [5].

Juniper cone berries are used in medicine, the food industry, as preservatives, and for the production of liquor/vodka beverages, in particular, the Slovak national drink "Borovichka". The essential oil is used in perfumery, insect repellents/insecticides, and microscopy as immersion oil cleaner. *Juniperus communis* contains monoterpenes, sesquiterpenes, essential and volatile oils, a wide range of phenolic compounds, and many other chemical constituents. In juniper cone berries, researchers found monoterpenes (α-pinene, α-cedrol, carene, α-terpinolen, and terpineol-4), sesquiterpenes (β-caryophyllene, Δ-cadinene, farnesol, γ-elemente, γ-muurolene, and humulene), and pregeijerene [6]. Cone berries also contain carbohydrates up to 30%, resins up to 10%, tannins up to 5%, flavonoids, bitterness, and organic acids (especially ascorbic). The nutritional value of juniper berries per 100 g is as follows: 19.0 g protein, 13.4 g fat, 33.0 g carbohydrates, 12.0 g fiber; energy value: 3.5 kcal/g or 353 kcal/1479 kg [6].

Due to the content of various biologically active substances in cone berries and juniper coniferous trees, it exerted many pharmacological effects, including antimicrobial, antiparasitic, antifertility, antioxidant, cytotoxic, hepatoprotective, vessel and trachea protective effects in passive smoking, gastrointestinal, antidiabetic, antihyperlipidemic, anti-inflammatory, analgesic, diuretic, antiurolithiatic, anti-Parkinsonian, memory enhancing, tyrosine suppressive activity, and many other effects [6].

One of the critical issues today is investigating the antioxidant action of *juniper* cone berries, which are part of beverages and food products and have a beneficial influence on human health. In raw plant materials, more than five thousand types of phytonutrients or non-living factors influence biochemical processes in the human organism. Their function is to slow down or disrupt unwanted oxidation reactions at the intracellular level. They can bind reactive forms of oxygen and other free radicals, reduce intermediate products of chain oxidation reactions, and weaken the activity of endogenous antioxidant enzymes. Phytonutrients have a high redox potential. However, the antioxidant property depends on the quantitative and qualitative composition of essential oils and phenolic derivatives, while the amount of carbohydrate, organic acid, and fat influences the quality of the distillate.

Juniper cone berries can be used as preservatives and antioxidant additives in the food industry. Previous investigations have demonstrated the activity of *J. communis* essential oil in inhibiting lipid oxidation and bacterial growth in cooked pork sausages. The results encourage further consideration of using *J. communis* essential oils as a healthier substitute for sodium nitrite in dry fermented sausages as an antioxidant additive [7].

The cone berries of *Juniperus drupace* and the Turkish national drink made from them, pekoes, contain phenolic compounds; pekoes is especially rich in protocatechuic acid, and the berries are rich in catechin and chrysin. Generally, phenolic levels decreased through simulated gastric conditions from the oral to the intestinal stage. The dialyzed fraction (IN) had the most significant antioxidant effect and was 0.77–12.19% of the initial values [8].

The purpose of our research was to establish the juniper growth sites with the best content of sugars, including fermentation, fats, organic acids, and the total phenol content of juniper cone berries and their antioxidant activity. The obtained indicators will make it possible to collect higher-quality raw materials for the production of the Slovak national drink "Borovychka" and the food industry as a whole.

2. Material and Methods

2.1. Plant Material and Habitats

The objects of the research were the shrub juniper blue (*Juniperus communis* L.) and juniper red (*Juniperus oxycedrus* L.)—representatives of the divisio *Pinophyta (Gymnosperma)*. To investigate the content of phenols and antioxidant activity, the collection of cone berries of *J. communis* was carried out in natural populations from Slovakia in 2016–2022 from the following locations: Spišský hrad, Kišovce—Hôrka, Chrámec Teplá dolina 1, Chrámec Teplá dolina 2, Chrámec Vlčia dolina 3, Chrámec Vlčia dolina 4, Cerovo, Selčianske sedlo, Lackov, Priechod-West, Priechod-East, Priechod-South, Liptovská Lužná 2, Liptovská Lužná 1, Liptovská Lužná 3, Horné lazy 2, Horné lazy 1, Pravica, Donovaly, Král'ová 1, Král'ová 2, Iliaš 2, Iliaš 1, as well as Žehra, Demiata, Devínska Kobyla, Dreveník, Horná Mariková, and Krasňany.

The collection of berry cones *J. communis* and *J. oxycedrus* was also investigated in other countries, such as Bulgaria (Plovdiv) and Albania (Elbasan, Bilisht). In addition, for research, berry cones of *J. communis* were purchased on the trade network of Bosnia, Serbia, and Czech (Hodonin), and cone berries of *J. communis* and *J. oxycedrus* were purchased from the Balkans (Albania).

The selected juniper cone berries were dried in dryers at a temperature of 38 °C for 6 h. The final moisture content of plant material for further processing was about 12%. Weighing of 100 juniper cones was repeated 6 times for each sample. Measurements were made using a Sartorius analytical scale of the CPA type.

2.2. Sugar Content

To determine the profile of sugars in juniper fruits, the method of highly efficient liquid chromatography (HPLC) (*European Pharmacopoeia 8.0*, 2.2.29. Liquid Chromatography) [4]. To determine the content of reducing carbohydrates, which is directed at determining the suitability of sugars during fermentation, the method of determining carbohydrates according to the Schorl method was tested [9].

2.3. Organic Acids

The quantitative content of the sum of organic acids was determined using potentiometric titration (methodology of the monograph *European Pharmacopoeia 8.0* "*Hibisci sabdariffae flos*" in terms of citric acid). Lipophilic fractions were received by exhaustive hexane extraction of cone berries in a Soxhlet apparatus for 1 h [4,10,11].

2.4. Total Phenolic Substances

Folin–Ciocalteu reagent is used to determine the total content of phenolic substances (TPS). Its principle consists in the reduction of the appropriate reagent, consisting of a mixture of phosphorous–tungstic acid and phosphorous–molybdic acid, to a mixture of blue oxides of tungsten and molybdenum by oxidation of phenols contained in the sample. The blue color shows maximum light absorption from 750 to 760 nm in the wavelength range. In contrast, the intensity of this absorption is directly proportional to the total amount of phenolic substances present. This is most often expressed in standard gallic acid equivalents (mg/L GAE) [12,13].

Methods for determining antioxidant potential or activity are based on the ability to eliminate (absorb) free radicals respectively on the oxidation–reduction properties of substances. Radicals can be formed in the reaction mixture, or they can be added to the reaction mixture. From a chemical point of view, these are oxygen radicals (hydroxyl, peroxyl, superoxide anion radical) or synthetic stable radicals (DPPH, FRAP, ABTS, galvinoxyl). The antioxidant property of juniper cone berries was determined by UV spectrophotometry using two radicals—DPPH and FRAP [12,14–16].

2.5. DPPH

A straightforward and rapid method commonly used to determine total antioxidant activity is the DPPH method, which uses the free, stable nitrogen radical of diphenylpicrylhydrazyl. Its principle is a reaction of the investigated substance with the stable synthetic radical diphenylpicrylhydrazyl (DPPH; 1, 1-diphenyl-2-(2, 4, 6-trinitrophenyl) hydrazyl), during which the radical is reduced to form DPPH-H (diphenylpicrylhydrazine). The DPPH neutralization test is based on donating electrons from antioxidants to neutralize the DPPH radical. Due to the intense coloring of this radical, the reaction is determined spectrophotometrically, most often at a wavelength of 515 nm, which corresponds to the maximum absorption of this radical. Using the DPPH method, only hydrophobic antioxidants that are soluble in organic solvents can be identified. The DPPH radical is sensitive to light, oxygen, and changes in pH, and results vary with different solvents [14,17].

2.6. FRAP

The FRAP (Ferric Reducing Ability of Plasma) method is a straightforward method based on evaluating the regenerating effects of antioxidants. Unlike other methods, no radical is used here. In this case, the antioxidants' reducing power is determined, not their ability to react with free radicals.

The FRAP method quantitatively determines the ability of antioxidants to reduce the colorless Fe (III)-TPTZ iron complex to blue Fe (II)-TPTZ (TPTZ = 2,4,6-tris(2-pyridyl-1,3,5-triazine). Increased absorption is measured at 593 nm and corresponds to the amount of Fe (II)-TPTZ complex, which is then a measure of the antioxidant activity of the sample. The results were expressed as μmol TE/g dw (mgM TE·100 g^{-1}). However, the method has its limitations, as the measurements are carried out at a physiologically low pH (3.6), and thus, slowly reacting polyphenolic components may not be captured by the complex. This method can determine the antioxidant activity of only hydrophilic compounds while the reaction is carried out in an acidic medium to maintain iron solubility [8,12,16].

2.7. Statistics

Determination of the content of carbohydrates, fats, organic acids, the total content of phenols in juniper cone berries, as well as their antioxidant activity was carried out in six replicates in plant material collected from each places of growth in Slovakia. The results were calculated using Microsoft Excel, version 7.0. As part of the analysis of the received data, several statistical methods and biometric parameters were used: the arithmetic mean; standard deviations—the error of the arithmetic mean (s_x) and the mean square deviation (σ); Student's t-criterion at the significance level of 0.05% and 0.01% (n = 6); and search analysis with a graphical representation of the frequencies of selected quantitative features using variation curves and diagrams. All studies were carried out in six-fold replication

Cluster analysis was performed using the program IBM SPSS Statistics 23 [18]. Populations with the highest, average, and lowest organic substance content were grouped using cluster analysis. The relationship between the content of phenols and their antioxidant effect was established. Also, with the help of cluster analysis, the juniper populations with the best sugar content, organic acids, fats, and antioxidant activity were determined, which can be a source of raw materials for the food industry [18].

3. Results

3.1. Organic Substances in Juniper Cone Berries in the Investigated Areas

The content of carbohydrates in raw materials collected from different growth areas was checked for the processing of juniper cone berries in the form of distillate by fermentation. Liquid chromatography was used to detect glucose and fructose (Table 1). Since these are renewable carbohydrates, the Schorl method was used to determine the course of fermentation maceration of juniper cone berries.

Table 1. Determination of the sugar content in juniper fruits by HPLC and Schorl methods.

Raw Material Juniper	Method HPLC		Schorl's Method	
	Fructose, g/kg of Dry Matter ($\bar{x} \pm s_X$) *	Glucose, g/kg of Dry Matter ($\bar{x} \pm s_X$)	Total, g/kg of Dry Matter ($\bar{x} \pm s_X$)	Reducing Sugars, g/kg of Dry Matter ($\bar{x} \pm s_X$)
Juniperus communis (Blue, Slovak)	173.1 ± 2.40	106.3 ± 2.40	279.4 ± 3.00	319.1 ± 3.20
J. oxycedrus (Red, Slovak)	85.6 ± 1.30	51.5 ± 0.85	137.1 ± 1.10	147.5 ± 1.85
J. communis (Blue, Albanian)	186.9 ± 2.50	187.7 ± 2.70	374.6 ± 2.70	360.0 ± 2.90
J. oxycedrus (Red, Albanian)	121.1 ± 1.20	84.0 ± 0.90	205.1 ± 2.50	197.4 ± 2.20

Legend: * $\bar{x}$—arithmetic mean; s_X—error of the arithmetic mean.

The results show that the content of restorative carbohydrates in blue juniper (*J. communis*) is higher than in red juniper (*J. oxycedrus*). Results also vary significantly between individual locations. This indicates that the conditions in which juniper cones ripen, and their species can affect the quality of the product. The total sugar and reducing sugar content was almost half higher in *J. communis* than in *J. oxycedrus*. The fructose content was 40% higher than glucose in both species of cone berries from Slovakia. Meanwhile, cone berries from Albania had almost the same amount of fructose and glucose in blue juniper and 30% less in red juniper.

To more comprehensively evaluate the quality of raw materials in terms of their organic substance content, juniper cone berries were collected from 31 places of growth in Slovakia, Bulgaria, and Albania in 2016 and 2017 (Table 2, Figure 1).

Table 2. Results of the analysis of samples of *Juniperus communis* and *Juniperus communis* cone berries for the content of carbohydrates, hexane-soluble components, organic acids, and weights of 100 juniper berries in different years and habitats.

№ Locality	Habitat/ Locality	Weight of Berries (100 Pieces), [g] ($\bar{x} \pm s_X$) *	Components Soluble in Hexane, % in Dry Matter ($\bar{x} \pm s_X$)	Carbohydrates, g/kg of Dry Matter ($\bar{x} \pm s_X$)	Organic Acids, g/kg of Dry Matter	Components Soluble in Hexane, % of Dry Mater ($\bar{x} \pm s_X$)	Carbohydrate, g/kg of Dry Matter ($\bar{x} \pm s_X$)	Organic Acids, g/kg of Dry Matter ($\bar{x} \pm s_X$)
			2016 Collection Year			2017 Collection Year		
1	Kišovce	5.84 ± 0.29	9.30 ± 0.30	41.60 ± 0.40	9.10 ± 0.03	10.50 ± 0.21	261.60 ± 14.09	11.80 ± 0.39
2	Liptovská Lúžna, 2	5.84 ± 0.16	10.40 ± 0.40	239.80 ± 2.40	7.40 ± 0.20	⌀	⌀	⌀
3	Liptovská Lúžná, 1	6.27 ± 0.37	10.00 ± 0.40	214.90 ± 2.10	8.80 ± 0.30	⌀	⌀	⌀
4	Selčianske sedlo	6.58 ± 0.07	10.30 ± 0.40	416.70 ± 4.20	12.50 ± 0.40	16.18 ± 0.32	281.69 ± 11.27	9.90 ± 0.99
5	Kráľová, 1	6.59 ± 0.09	14.60 ± 0.50	297.90 ± 3.00	12.80 ± 0.40	15.88 ± 0.32	309.97 ± 12.40	11.40 ± 1.14
6	Liptovská Lúžna, 3	6.87 ± 0.08	11.30 ± 0.40	269.40 ± 2.70	10.30 ± 0.30	⌀	⌀	⌀
7	Horné Lazy, 2	7.22 ± 0.04	12.00 ± 0.30	384.80 ± 3.80	5.20 ± 0.20	15.84 ± 0.32	421.56 ± 16.86	10.10 ± 1.01
8	Kráľová, 2	7.43 ± 0.19	14.70 ± 0.50	270.90 ± 2.70	11.40 ± 0.40	⌀	⌀	⌀
9	Donovaly	7.53 ± 0.21	8.60 ± 0.30	403.50 ± 4.00	8.60 ± 0.30	⌀	⌀	⌀

Table 2. *Cont.*

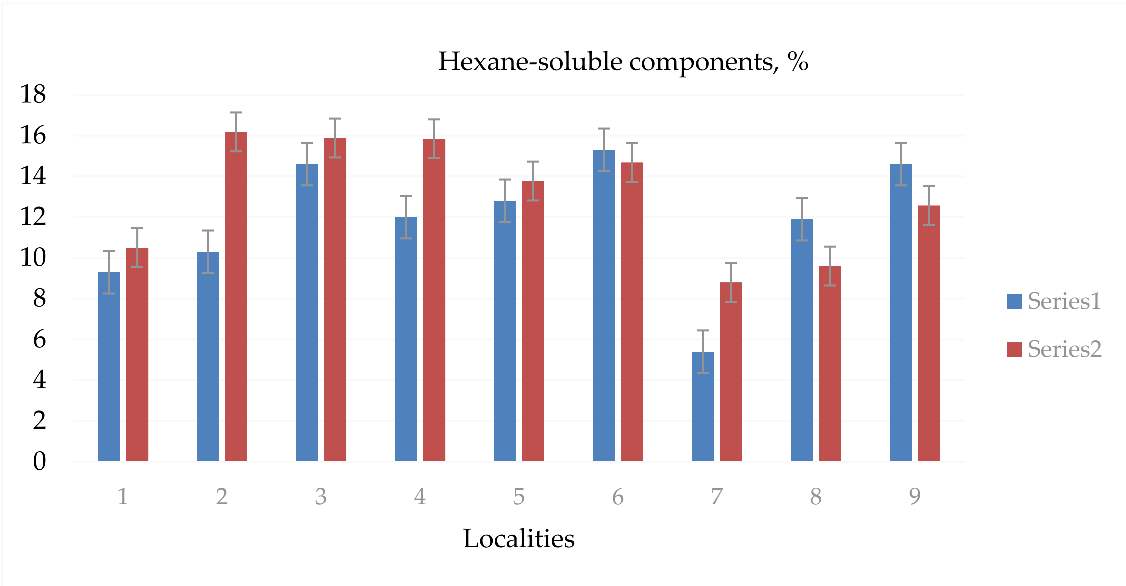
№ Locality	Habitat/ Locality	Weight of Berries (100 Pieces), [g] ($\bar{x} \pm s_X$) *	Components Soluble in Hexane, % in Dry Matter ($\bar{x} \pm s_X$)	Carbohydrates, g/kg of Dry Matter ($\bar{x} \pm s_X$)	Organic Acids, g/kg of Dry Matter	Components Soluble in Hexane, % of Dry Mater ($\bar{x} \pm s_X$)	Carbohydrate, g/kg of Dry Matter ($\bar{x} \pm s_X$)	Organic Acids, g/kg of Dry Matter ($\bar{x} \pm s_X$)
			2016 Collection Year			2017 Collection Year		
10	Cerovo	7.83 ± 0.26	14.10 ± 0.50	300.20 ± 3.00	8.60 ± 0.30	⍁	⍁	⍁
11	Iliaš, 1	8.07 ± 0.13	8.30 ± 0.30	273.30 ± 2.70	7.70 ± 0.20	⍁	⍁	⍁
12	Priechod-West	8.09 ± 0.17	8.50 ± 0.30	330.10 ± 3.30	13.30 ± 0.40	⍁	⍁	⍁
13	Priechod-South	8.33 ± 0.23	11.50 ± 0.40	304.10 ± 3.00	13.00 ± 0.40	⍁	⍁	⍁
14	Spišsky hrad	8.44 ± 0.23	11.60 ± 0.40	371.20 ± 3.70	12.70 ± 0.40	⍁	⍁	⍁
15	Priechod-East	8.69 ± 0.20	12.90 ± 0.50	307.90 ± 3.10	8.60 ± 0.30	⍁	⍁	⍁
16	Horné Lazy	9.18 ± 0.08	12.80 ± 0.50	403.80 ± 4.00	10.20 ± 0.30	13.77 ± 0.28	566.16 ± 22.65	10.44 ± 1.04
17	Lackov	9.24 ± 0.08	15.30 ± 0.60	265.70 ± 2.70	11.80 ± 0.40	14.68 ± 0.29	314.03 ± 12.56	14.50 ± 1.45
18	Iliaš 2	11.65 ± 0.10	10.20 ± 0.40	315.90 ± 3.20	8.00 ± 0.30	⍁	⍁	⍁
19	Chrámec Teplá dolina 1	10.35 ± 0.16	⍁	⍁	⍁	10.70 ± 0.21	367.50 ± 17.00	10.00 ± 0.34
20	Chrámec Teplá dolina 2	15.08 ± 0.34	⍁	⍁	⍁	11.90 ± 0.24	344.00 ± 15.89	12.70 ± 0.44
21	AL, Elbasan, *J.oxycedrus*	⍁	5.40 ± 0.20	208.80 ± 2.10	3.40 ± 0.10	8.80 ± 0.18	321.70 ±14.86	7.90 ± 0.27
22	AL, Elbasan, *J.communis*	⍁	11.90 ± 0.40	375.90 ± 3.80	6.90 ± 0.20	9.60 ± 0.19	165.40 ± 7.64	3.40 ± 0.11
23	Dreveník	⍁	14.60 ± 0.50	316.60 ± 3.20	9.70 ± 0.30	12.57 ± 0.25	320.25 ± 12.81	12.19 ± 1.22
24	Chrámec—Teplá dolina	⍁	11.00 ± 0.40	333.80 ± 3.30	11.40 ± 0.40	⍁	⍁	⍁
25	Chrámec—Vlčia dolina	⍁	13.60 ± 0.50	376.60 ± 3.80	15.00 ± 0.50	⍁	⍁	⍁
26	BG, Plovdiv, *J. communis*	⍁	6.70 ± 0.20	381.60 ± 3.80	10.90 ± 0.30	⍁	⍁	⍁
27	BG, Plovdiv, *J. oxycedrus*	⍁	4.70 ± 0.20	351.20 ± 3.50	2.50 ± 0.10	⍁	⍁	⍁
28	AL, Bilisht, *J. oxycedrus*	⍁	13.70 ±0.50	371.80 ± 3.70	7.40 ± 0.20	⍁	⍁	⍁
29	AL, Bilisht, *J.communis*	⍁	10.60 ± 0.40	76.10 ± 0.80	7.20 ± 0.20	⍁	⍁	⍁
30	Horná Mariková	⍁	17.80 ± 0.60	309.40 ± 3.10	11.80 ± 0.40	⍁	⍁	⍁
31	Žehra	⍁	12.10 ± 0.40	287.20 ± 2.90	10.60 ± 0.30	⍁	⍁	⍁
32	Demiata	⍁	13.40 ± 0.50	388.10 ± 3.90	13.20 ± 0.40	⍁	⍁	⍁
33	Devínska Kobyla	⍁	13.3 ± 0.5	383.7 ± 3.8	11.2 ± 0.4	⍁	⍁	⍁
34	Vlčia dolina-východ 1	⍁	⍁	⍁	⍁	11.20 ± 0.22	347.60 ± 16.06	11.20 ± 0.38
35	Vlčia dolina-západ	⍁	⍁	⍁	⍁	9.40 ± 0.19	359.90 ± 16.63	10.50 ± 0.35
36	Krasňany	⍁	⍁	⍁	⍁	11.96 ±0.24	418.73 ± 16.75	10.03 ± 1.00
37	Mariková	⍁	⍁	⍁	⍁	12.97 ± 0.26	352.90 ± 14.12	10.28 ± 1.03
38	Iliaš	⍁	⍁	⍁	⍁	12.89 ± 0.26	364.75 ± 14.59	15.20 ± 1.52
39	Ostrá hora	⍁	⍁	⍁	⍁	14.20 ± 0.28	228.90 ± 10.58	19.90 ± 0.63
40	Priechod	⍁	⍁	⍁	⍁	13.25 ± 0.27	323.85 ± 12.95	12.90 ± 1.29

Legend: ⍁—the analyses were not conducted; * $\bar{x}$—arithmetic mean; s_X—error of the arithmetic mean.

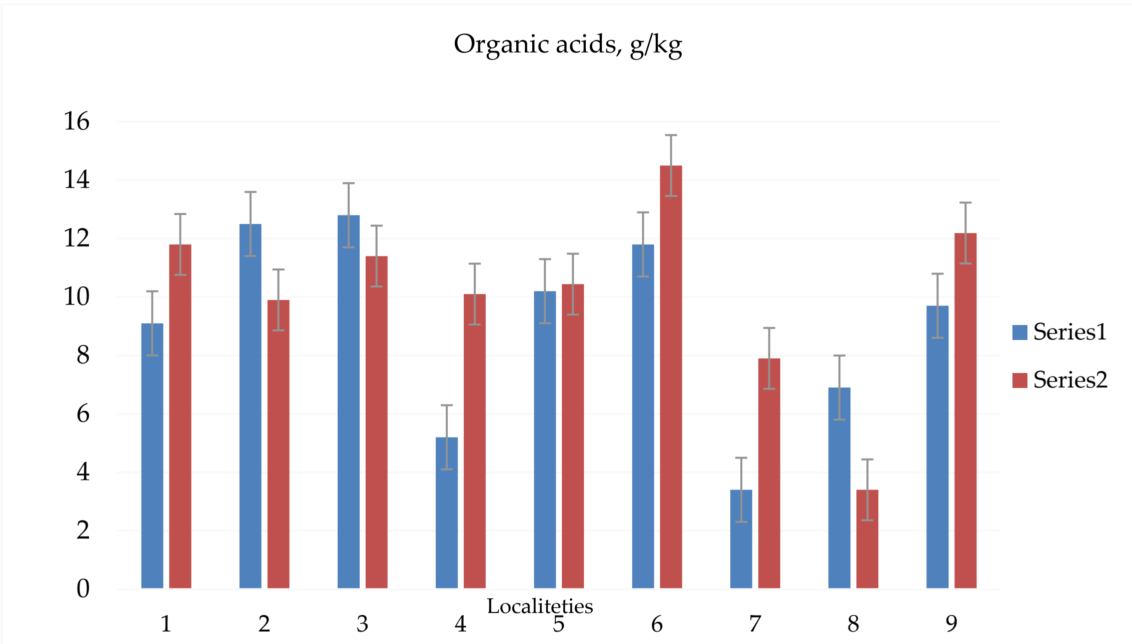
The results differ significantly between individual places of selection. The sugar content in cone berries harvested in 2016 ranged from 41.6 ± 0.4 g/kg (locality Kišovce) to 416.7 ± 4.2 g/kg (locality Selčianske sedlo). In 2017, the carbohydrate content of Junipers in Kišovce was 261.6 ± 14.09, while in Selčianske sedlo, it was 35% less than in the previous year. In 2017, the lowest quantity of sugar components was recorded in the Albanian sample (165 ± 7.64 g/kg) and was the highest in the sample from the Horné Lazy area (566.16 ± 22.65 g/kg), against 403.8 ± 4.0 and 375.9 ± 3.8, respectively, in the previous year (Figure 1C). In summary, we can conclude that the sugar content in juniper that grows in Slovakia is more than 300 g/kg. In the sample, *J. oxycedrus* was found to have lower carbohydrate content than *J. communis*.

Many collected Juniperus samples also presented exciting results regarding the content of organic acids. Their content ranges from 2.5 ± 0.1 g/kg to 13.3 ± 0.4 g/kg of berries. All samples of both types of *Juniperus* from the Balkans contained deficient levels of organic acids in both years of the investigation. Most juniper cone berry samples from Slovakia had values higher than 10.0 g/kg. The fat content was low and ranged from 8.8 ± 0.18 to 16.18 ± 0.32 percent of the dry matter of *Juniperus* cone berries.

Moreover, the fat content was somewhat higher in the second year (2017) (Table 2, Figure 1A). The fat content can be considered stable based on the variability expressed by the confidence interval. Also, *J. oxycedrus* has a significantly lower content of organic acids and fats than *J. communis*.

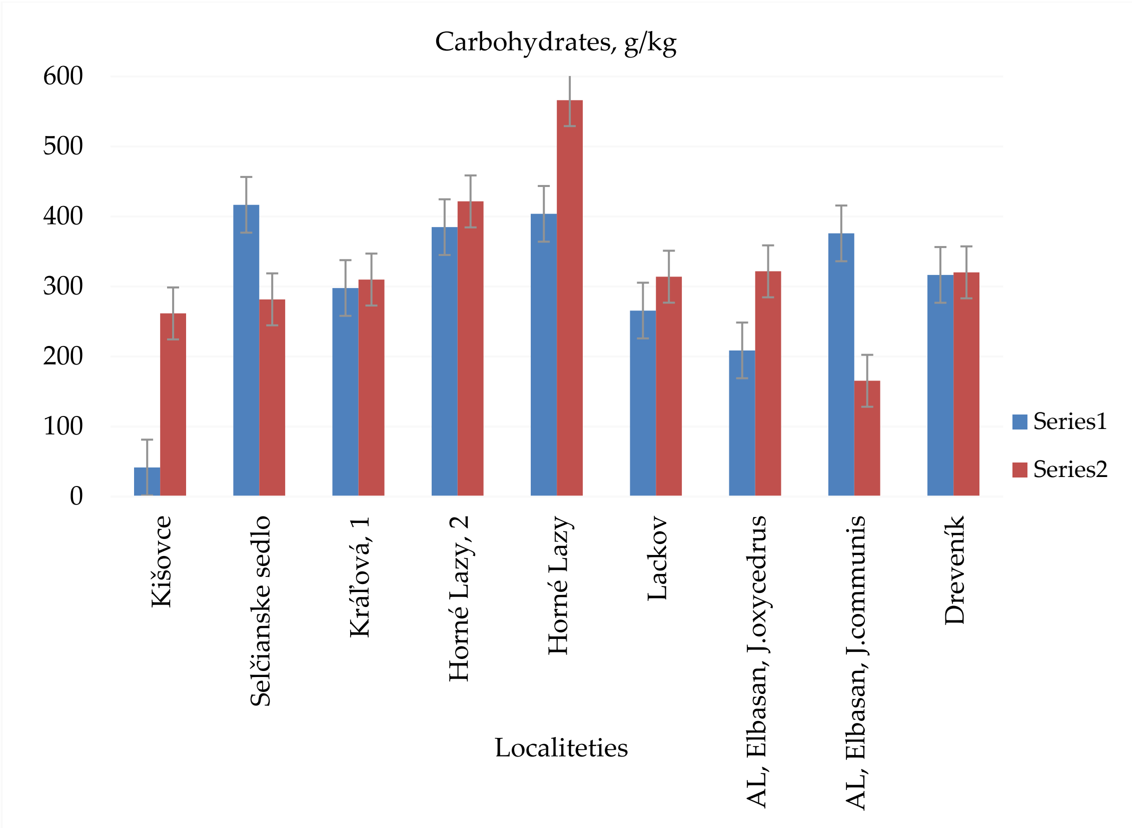


(**A**)



(**B**)

Figure 1. *Cont*.



(**C**)

Figure 1. The content of hexane-soluble components, organic acids, and carbohydrates in cone berries of *J. communis* from places of growth in different years: (**A**) Hexane-soluble components, %; (**B**) Organic acids, g/kg; (**C**) Carbohydrates, g/kg. Series 1—2016 Collection Year; Series 2—2017 Collection Year.

As can be seen from the diagram (Table 2, Figure 1), the content of organic substances in Juniperus cone berries varied greatly from year to year and mainly depends on their maturity and weather conditions in the year of collection. At full maturity, maximum sugar content and quite large sizes (9.0 to 15.0 g) are achieved. The fruits are dark blue in color and contain a sufficient quantity of nutrients; the seeds are ripe, and the fruit falls off more easily. At this maturity, it is necessary to optimize the collection of raw materials, because such parameters are the best for fermentation at optimal pH and temperature.

3.1.1. Cluster Analysis of Juniper Fruit Weight Relationships from Locations and Their Content of Organic Substances

The definition of the best place for growth of *J. communis* with the most optimal content of organic substances in cone berries was carried out using cluster analysis, which compared the results received in 2016 from 1–20 localities (Table 1).

Because of the conducted cluster analysis, five groups of *J. communis* populations growing in Slovakia were set based on the ratio of weight of 100 juniper cone berries to organic substance content: fats, carbohydrates, and organic acids. The results are reduced to percentages of the highest value in the sample (Figure 2).

Based on the diagram and table of the distribution of agglomerations (clusters) using the cluster similarity coefficient, we grouped the digital material (seed weight, content of fats, sugars, organic acids) into five groups (Figure 2).

First group: Cone berries were collected from Liptovská Lúžná 1, Liptovská Lúžna 2, Liptovská Lúžna 3, Donovaly, Iliaš 1, and Horné Lazy 2 populations and had low seed weight, medium fat content, medium-high carbohydrate content, and low-medium organic acid content (39–54:56–78:58–97:39–77%).

Second group: Juniperus from the Priechod-South, Priechod-West, Spišsky hrad, and Selčianske sedlo populations had an average seed weight of 44–56 and an average fat content of 56–76. They also had medium-high carbohydrate and organic acid content (73–100 and 95–100, respectively).

Third group: In the population of Kráľová, 1; Kráľová, 2; Cerovo, Horné Lazy, Priechod-East, Lackov cone berries had an average weight, high-fat content, and medium-high carbohydrates and organic acids content (44–61:84–100:64–97:65–96%).

Fourth group: Iliaš 2 populations should be separated into separate groups: Chrámec Teplá dolina 1 and Chrámec Teplá dolina 2 cone berries that had large seeds but medium-high content of organic substances: 69–100:67–78:76–88:60–95%.

Fifth group: One Kišovce population has tiny seeds, very low carbohydrate content, and medium content of other substances (39:61:10:68%).

Other clusters are irrelevant.

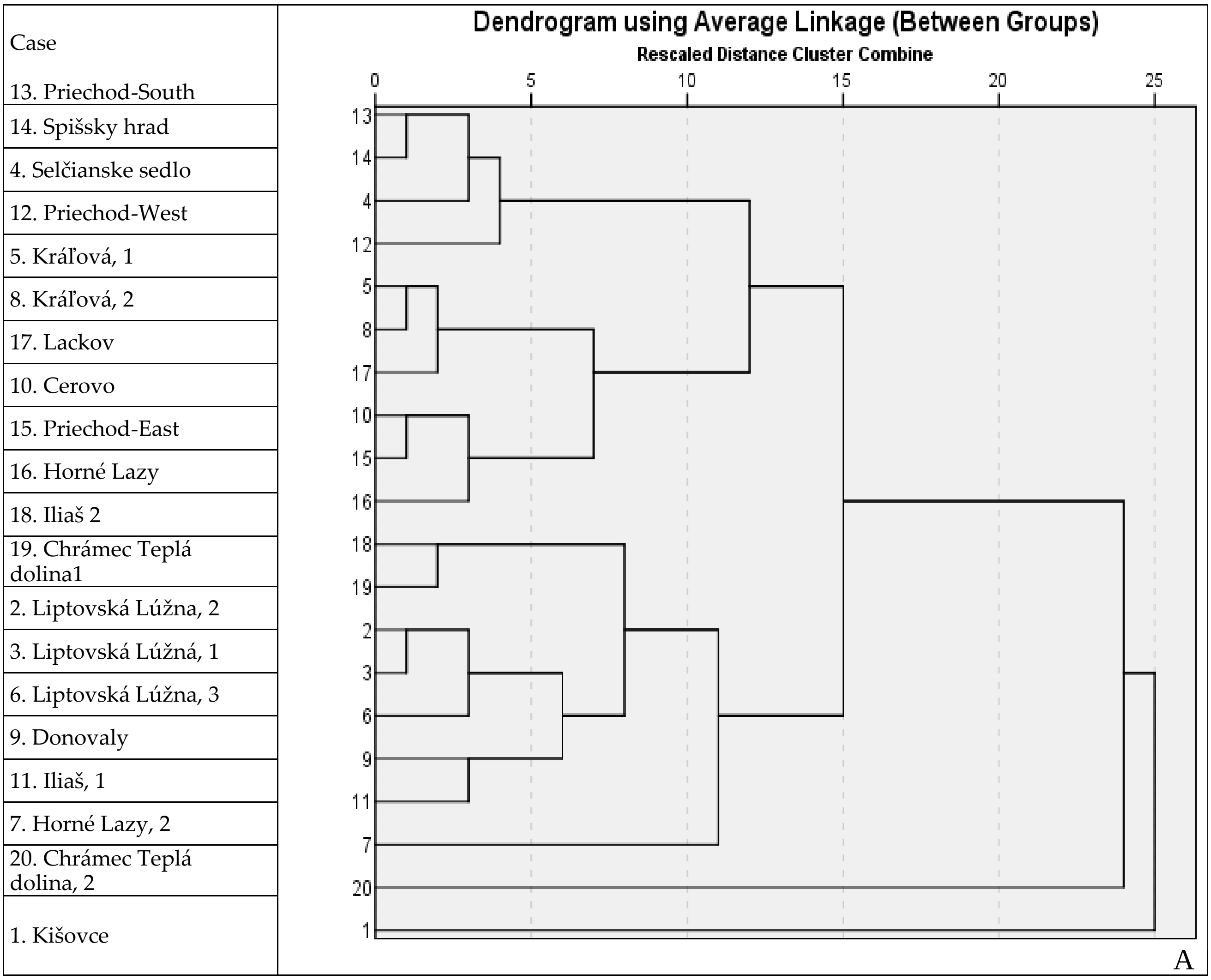


Figure 2. *Cont*.

Table of Agglomeration Schedule (B)

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	13	14	0.035	0	0	7
2	2	3	0.038	0	0	8
3	5	8	0.039	0	0	5
4	10	15	0.040	0	0	9
5	5	17	0.079	3	0	13
6	18	19	0.100	0	0	14
7	4	13	0.125	0	1	11
8	2	6	0.125	2	0	12
9	10	16	0.137	4	0	13
10	9	11	0.138	0	0	12
11	4	12	0.178	7	0	16
12	2	9	0.257	8	10	14
13	5	10	0.264	5	9	16
14	2	18	0.337	12	6	15
15	2	7	0.438	14	0	17
16	4	5	0.484	11	13	17
17	2	4	0.571	15	16	18
18	2	20	0.910	17	0	19
19	1	2	0.985	0	18	0

Figure 2. Dendrogram of cluster analysis (**A**) and table of schedule of agglomerations (**B**) of *Juniperus communis* populations from different growth locations by weight of 100 pieces of cone berry and organic matter content (fats (hexane-soluble components), carbohydrates, organic acids). The results are reduced to percentages of the highest value in the sample.

3.1.2. Weight of Juniper Fruits and Contents of Organic Substances Depending on the Geographical Location of *Juniperus communis* Populations

As shown in Figure 1, the content of organic substances in Juniperus cone berries varied considerably from year to year. It mainly depended on their maturity, growth, and weather conditions in the collection year. The maximum sugar content and largest sizes are achieved at full maturity. The dark blue fruits contain sufficient nutrients, the seeds are ripe, and the fruit falls off more easily. At this maturity, it is necessary to optimize the collection of raw materials because such parameters are the best for fermentation at optimal pH and temperature. Graphically, the weight of seeds and the content of organic substances are related to the geographical location of the population, as shown in Figure 3.

As for the influence of environmental conditions on the quality of Juniperus cone berries, plants growing in the Carpathian lowlands on the northern side (populations Chrámec Teplá dolina 1, Iliaš 2, and Chrámec Teplá dolina 2) had the most seeds (10–15 g the weight of 100 cone berries). They had average carbohydrate content and above-average fat and organic matter. The size of the cone berries decreased with the ascent to the mountains (Table 3, group 5; Figure 3).

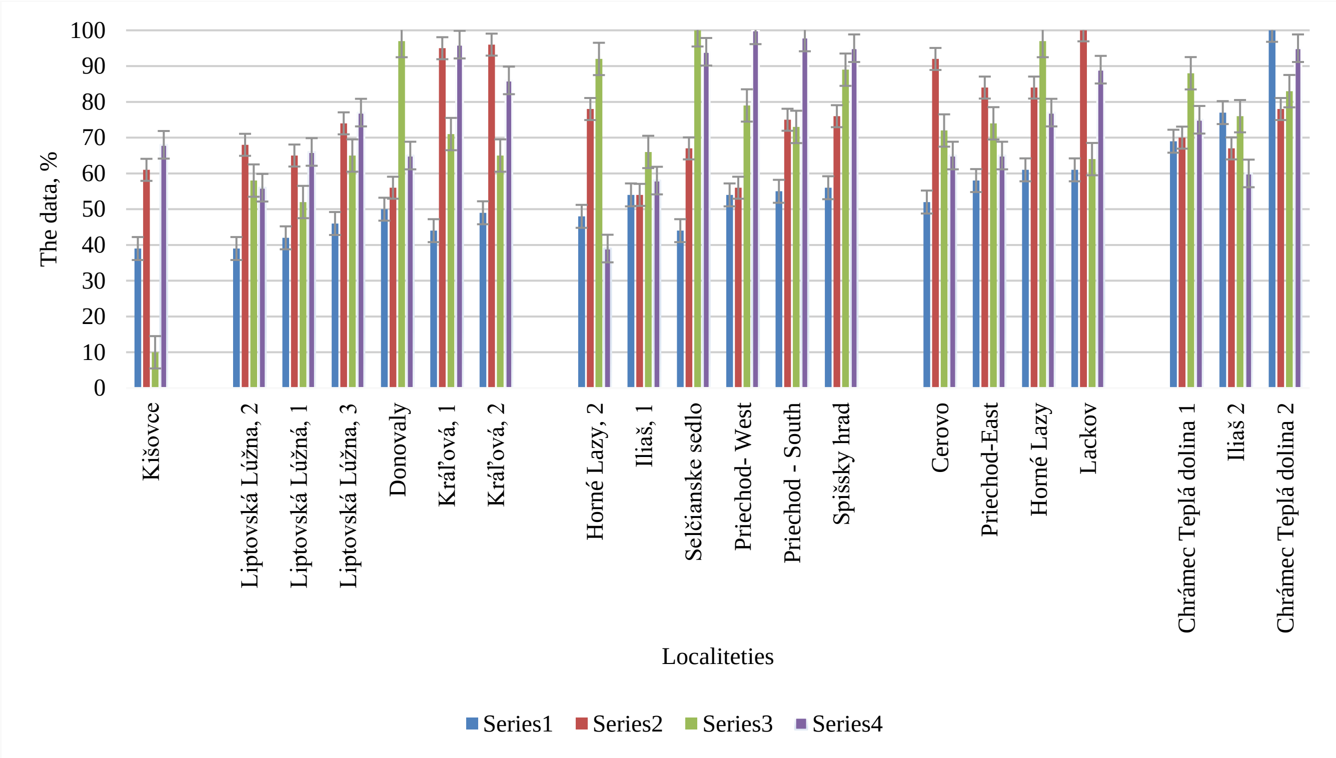


Figure 3. The weight, content of carbohydrates, organic acids, and hexane-soluble components in cone berries of *J. communis* from places of growth in Slovakia, collected in groups, depending on geographic distribution: series 1—weight 100 pieces of cone berries (in %); series 2—the content (in %) of hexane-soluble components; series 3—the content (in %) of carbohydrates; series 4—the content (in %) of organic acids. The results are reduced to percentages of the highest value in the sample.

The highest content of organic substances—fats, carbohydrates, and proteins—was found in plants at medium altitudes (Priechod-South, Priechod-West, and Spišsky hrad); only plants from the places of growth Cerovo, Horné Lazy, Priechod-East, and Lackov had average fat content. Cone berries from these growth places were of average size (8.09–9.24 g the weight of 100 cone berries) (Table 3, groups 3, 4). The exceptions are the populations Iliaš 1, Horné Lazy 2, and Selčianske sedlo, which grow on the northern slope of medium altitudes; they have average seed weight (6.58–8.07 g the weight of 100 cone berries), sugar, and fat content but a low content of organic acids.

Juniperus cone berries collected from Liptovská Lúžná 1, Liptovská Lúžna 2, Liptovská Lúžna 3 (above 700 m above sea level), Donovaly, Iliaš 1, and Horné Lazy 2 (Table 3, group 1, 2) had tiny seeds (5.84–7.53 g the weight of 100 cone berries) and low organic matter content. Populations Kráľová, 1 and Kráľová, 2, which are located 1000 m above sea level, had small fruits, with high content of fats and organic acids, but less sugars. The content of organic substances, especially sugars, was low in cone berries from Kišovce (northern slope) (Table 2, Figure 3).

The content of organic substances was weakly correlated with the weight of 100 fruits from natural populations of *J. communis* in Slovakia. Thus, large fruits collected from plants on the lower, northern slopes of the mountains (Chrámec Teplá dolina 1, Chrámec Teplá dolina 2, and Iliaš 2) had the most significant seed weight (10–15 g the weight of 100 cone berries). However, they had an average content of organic substances. At the same time, the medium-sized cone berries were collected at medium altitudes (Priechod-South, Priechod-West, Spišsky hrad, Selčianske sedlo). They had a relatively high content of all the investigated organic substances. Only plants from the places of growth Kráľová, 1; Kráľová, 2; Cerovo, Horné Lazy, Priechod-East, Lackov had an average fat content. Juniper cone berries collected in the places of growth Liptovská Lúžná 1 growing area; Liptovská

Lúžna 2, Liptovská Lúžna 3 (above 700 m above sea level), Donovaly, Iliaš 1 and Horné Lazy 2, especially Kišovce had tiny seeds and low organic matter content.

The highest seed weight (10–15 g the weight of 100 cone berries) had the fruits of plants in the Carpathian lowlands on the northern side, but they had an average content of organic substances. The size of cone berries and their content of organic substances decreased with the ascent to the mountains. The best content of organic substances was found in plants at medium altitudes.

Table 3. The weight 100 pieces of cone berry and the quantity of organic substances depend on the geographical location of *Juniperus communis* populations.

Habitat/Locality	Weight of Berries (100 Pieces), [g] ($\bar{x} \pm s_X$) *	Soluble Components in Hexane, g/kg is in Dry Matter ($\bar{x} \pm s_X$)	Sugars, g/kg of Dry Matter ($\bar{x} \pm s_X$)	Organic Acids, g/kg of Dry Matter ($\bar{x} \pm s_X$)	Geographical Latitude	Terrestrial Longitude	Altitude [m]	Aspect	Slope
1st group									
Kišovce	5.84 ± 0.29	9.30 ± 0.30	41.60 ± 0.40	9.10 ± 0.30	N 49°02′84″	E 20°38′11″	620	Northeast	30°
2nd group									
Liptovská Lúžna, 2	5.84 ± 0.16	10.40 ± 0.40	239.80 ± 2.40	7.40 ± 0.20	N 48°56′30″	E 19°19′12″	700	South	15°
Liptovská Lúžna, 3	6.87 ± 0.08	11.30 ± 0.40	269.40 ± 2.70	10.30 ± 0.30	N 48°56′29″	E 19°19′13″	710	Southeast	35°
Liptovská Lúžná, 1	6.27 ± 0.37	10.00 ± 0.40	214.90 ± 2.10	8.80 ± 0.30	N 48°56′31″	E 19°19′15″	730	Southwest	25°
Donovaly	7.53 ± 0.21	8.60 ± 0.30	403.50 ± 4.00	8.60 ± 0.30	N 48°52′48″	E 19°13′21″	960	North	35°
Král'ová, 2	7.43 ± 0.19	14.70 ± 0.50	270.90 ± 2.70	11.40 ± 0.40	N 48°52′51″	E 20°08′25″	1010	South	25°
Král'ová, 1	6.59 ± 0.09	14.60 ± 0.50	297.90 ± 3.00	12.80 ± 0.40	N 48°52′58″	E 20°08′21″	1272	East	35°
3rd group									
Iliaš, 1	8.07 ± 0.13	8.30 ± 0.30	273.30 ± 2.70	7.70 ± 0.20	N 48°41′55″	E 19°18′37″	340	Northeast	12°
Horné lazy 2	7.22 ± 0.04	12.00 ± 0.30	384.80 ± 3.80	5.20 ± 0.20	N 48°48′51″	E 19°36′00″	475	Northeast	14°
Selčianske sedlo	6.58 ± 0.07	10.30 ± 0.40	416.70 ± 4.20	12.50 ± 0.40	N 48°45′53″	E 19°12′24″	380	Northeast	10°
Priechod-South	8.33 ± 0.23	11.50 ± 0.40	304.10 ± 3.00	13.00 ± 0.40	N 48°46′41″	E 19°13′49″	420	South	15°
Priechod-West	8.09 ± 0.17	8.50 ± 0.30	330.10 ± 3.30	13.30 ± 0.40	N 48°46′45″	E 19°13′54″	480	West	20°
Spišsky hrad	8.44 ± 0.23	11.60 ± 0.40	371.20 ± 3.70	12.70 ± 0.40	N 49°00′00″	E 20°46′06″	628	South	30°
4th group									
Priechod-East	8.69 ± 0.20	12.90 ± 0.50	307.90 ± 3.10	8.60 ± 0.30	N 48°46′40″	E 19°13′50″	390	East	10°
Cerovo	7.83 ± 0.26	14.10 ± 0.50	300.20 ± 3.00	8.60 ± 0.30	N 48°15′17″	E 19°09′26″	468	Northwest	21°
Lackov	9.24 ± 0.08	15.30 ± 0.60	265.70 ± 2.70	11.80 ± 0.40	N 48°19′15″	E 19°11′12″	476	Southeast	16°
Horné Lazy	9.18 ± 0.08	12.80 ± 0.50	403.80 ± 4.00	10.20 ± 0.30	N 48°48′51″	E 19°36′50″	520	Northeast	30°
5th group									
Chrámec Teplá dolina 1	10.35 ± 0.16	10.70 ± 0.21	367.50 ± 17.00	10.00 ± 0.34	N 48°15′35″	E 20°10′56″	248	Northeast	22°
Iliaš 2	11.65 ± 0.10	10.20 ± 0.40	315.90 ± 3.20	8.00 ± 0.30	N 48°41′52″	E 19°18′32″	320	East	16°
Chrámec Teplá dolina 2	15.08 ± 0.34	11.90 ± 0.24	344.00 ± 15.89	12.70 ± 0.44	N 48°15′34″	E 20°10′60″	196	South	5°

Legend: * $\bar{x}$—arithmetic mean; s_X—error of the arithmetic mean.

3.2. Total Phenolic Compounds and Antioxidant Activity of Juniper Berries Determined by the DPPH and FRAP Methods—International Samples

Content of total phenolics was determined by FC method. Raw materials were purchased in trade networks and collected from natural habitats in various European countries. The raw materials for the research were cone berries *Juniperus communis* from the trade network of Bosnia, Serbia, the Czech Republic (Hodonin), and Albania, as well as cone berries of *J. oxycedrus* from Albania. Determination of the content of phenols in juniper cone berries and their antioxidant activity was carried out six times. Average values and their standard deviations were calculated on the basis of research.

In further investigations, the content of phenols and the antioxidant activity of *Juniperus* cone berries collected in separate natural populations in Slovakia were studied.

First, the number of phenolic substances (total phenolic content, TPC) was determined since plant phenols (phenolic acids and their derivatives, tannins, coumarin derivatives, flavonoids, isoflavonoids, prenylated flavonoids, stilbene derivatives, etc.) belong to substances with a high ability to absorb free radicals. Then, the antioxidant activity of alcoholic extracts of *Juniperus* cone berries was determined by the DPPH and FRAP methods.

Table 4 shows the results of determining the content of phenolic compounds in *Junipers* cone berries. As a result of the conducted research, it was observed that the amount of TPC was different. The highest content was recorded in the extract of the cone berries of *J. communis* in a commercial sample from Serbia (417.27 mg GAE·100 mL^{-1}), with a slightly lower content (365.91 mg GAE·100 mL^{-1}) in cone berries from Albania. The lowest (205.45 mg GAE·100 mL^{-1}) was in a commercial sample from Bosnia. A relatively low content of phenols was observed in *J. oxycedrus* (269.09 mg GAE·100 mL^{-1}).

Table 4. Determination of the content of phenolic compounds, DPPH and FRAP methods, and antioxidant activity of cone berries of *Juniperus* species from different countries.

Place of Origin	**TPC**	**DPPH**	**FRAP**
	mg GAE·100 g^{-1} ($\bar{x} \pm s_X$) *	**% ($\bar{x} \pm s_X$)**	**mgM TE·100 g^{-1} ($\bar{x} \pm s_X$)**
Bosnia—trade network—*J. communis*	205.45 ± 0.226	35.46 ± 0.555	1236.96 ± 0.285
Serbia—trade network—*J. communis*	417.27 ± 0.459	63.37 ± 0.315	2950.00 ± 0.679
Czech Republic (Hodonín)—*J. communis*	215.00 ± 0.237	31.16 ± 0.592	1426.09 ± 0.328
Albania—*J. communis*	365.91 ± 0.403	51.04 ± 0.421	2408.70 ± 0.554
Albania—*J. oxycedrus*	269.09 ± 0.296	39.41 ± 0.521	2086.96 ± 0.480

Legend: * $\bar{x}$—arithmetic mean; s_X—error of the arithmetic mean.

The antioxidant activity was determined by the DPPH method, which uses the free, stable nitrogen radical of diphenylpicrylhydrazyl and the FRAP (Ferric Reducing Ability of Plasma), based on evaluating the regenerating effects of antioxidants.

The DPPH method is considered one of the main methods of evaluating the antiradical activity of pure substances and their various mixtures. From the measurement results in Table 4, the *J. communis* cone berry samples from Serbia and Albania showed the highest antioxidant activity. Accordingly, it corresponds to the high absorption (63.37 and 51.04%) of the synthetic diphenylpicrylhydrazyl radical by juniper alcohol extract.

The FRAP chemical method was used to determine the antioxidant activity in the second case for *Juniperus* of different origins sold on an industrial scale (Table 4). The results were expressed as mgM TE·100 g^{-1}.

The highest values of antioxidant potential were achieved by a sample of cones of *J. communis* from Serbia (2950.00 mgM TE·100 g $^{-1}$) and Albania (2408.70 mgM TE·100 g $^{-1}$).

As shown in Figure 4, the higher the content of phenols in juniper cone berries, the higher their antioxidant activity. Thus, the industrial sample of *J. communis* from Serbia and Albania had the highest content of phenols (417.21, and 365.91 mg GAE 100 g^{-1}, respectively). For ease of comparison, the data have been converted into percentages. Thus, the content of phenols and antioxidant capacity in the industrial sample of *J. communis* from Serbia was the highest and amounted to 100%, with that from Albania being 12–20% less. These plants had the highest antioxidant activity (63.37, and 51.04% by the DPPH method, and 29,500 and 2408.7 mgM TE·100 g^{-1} by the FRAP method). In other investigated species—*J. oxycedrus* from Albania and *J. communis* from the Czech Republic and Bosnia—the content of phenols and antioxidant activity was 40–50% lower.

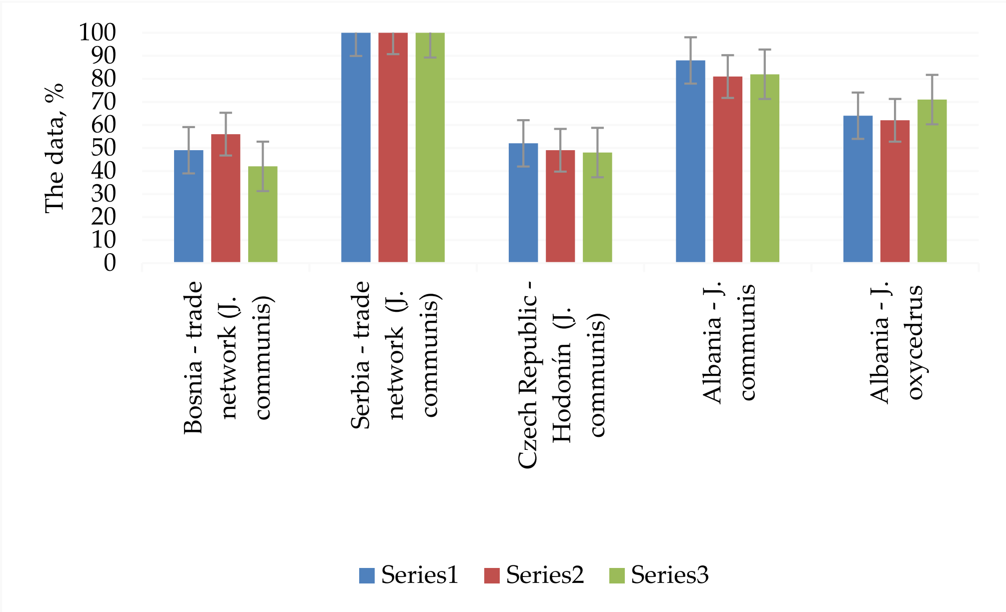


Figure 4. Dependence of the antioxidant action on the content of phenols in various species of the genus *Juniperus*, purchased on the trade network: Series 1—content of phenols (%); Series 2—antioxidant activity of cone berries, determined by the DPPH method (%); Series 3—and antioxidant activity of *Juniperus* cone berries, determined by the FRAP method (%).

3.3. Total Phenolic Compounds and Antioxidant Activity of Juniper Berries Determined by DPPH and FRAP Methods—Samples from Slovakia

The collection of *J. communis* cone berries from natural populations in Slovakia with different ecological conditions was carried out. The results may become important for further investigations of *Juniperus* cone berries for pharmacological use and in the food industry for use as preservatives or for extending the term of raw and processed food products.

The results of determining the total amount of phenolic substances (TPS) were selected from several analyses of *Juniperus* samples selected in different growth places (Table 5). The highest phenol content was recorded in samples from Chrámec Teplá dolina (181.68 ± 3.149 mg GAE·100 mL^{-1}), Horné lazy (172.07 ± 1.148 mg GAE·100 mL^{-1}), and Iliaš (166.82 ± 1.932 mg GAE·100 mL^{-1}). The lowest TPC content was found in samples from Chhrámec–Vlčia dolina (50.15 ± 2.381 mg GAE per 100 mL^{-1} and 93.54 ± 2.112 mg GAE per 100 mL^{-1}) and Pravica (95.20 ± 3.196 mg GAE per 100 mL^{-1}). From the review of the determined amounts of phenols with their statistical evaluation (n = 6) in some places of Slovakia, it is clear that the course of their biosynthesis depends on abiotic and biotic conditions. At the same time, we can generalize that the content of TRS in industrial samples is significantly different compared to those collected from natural habitats in Slovakia. In the first part of the section on the antioxidant activity of *Juniperus*, it was reported that the amount of TPC in the extract of *Juniperus* blue cones from Albania is up to 365.91 mg GAE per 100 mL^{-1} and from Serbia, an even higher 417.27 mg GAE per 100 mL^{-1}. While naturally, the phenolic content was almost half that of best sample (Chrámec Teplá dolina (181.68 ± 3.149 mg GAE·100 mL^{-1}). The probable reason is the origin of the sample and its purpose; better and larger ones were selected for the trade network based on the size of the cone berries intended for food consumption. The cone berries of *J. oxycedrus* also had a high content of phenols (269.09 mg GAE·100 mL^{-1}).

Table 5. Content of phenolic compounds (TPC), DPPH, and FRAP method and determination of antioxidant potential [AA, %] of fruits collected from *Juniperus communis* populations in Slovakia.

№	Locations	TPC [mg GAE·100 mL^{-1}]		DPPH Method [AA, %]		FRAP Method [mgM TE·100 g^{-1}]	
	n = 6	($\bar{x} \pm s_X$) *	$\bar{x} \pm t.s_X$ ** [0.01%]	$\bar{x} \pm s_X$	$\bar{x} \pm t.s_X$ [0.01%]	$\bar{x} \pm s_X$	$\bar{x} \pm t.s_X$ [0.01%]
1.	Spišský hrad, Ostrá hora	103.60 ± 1.4896	103.60 ± 2.452	15.04 ± 0.2888	15.04 ± 0.475	1972.12 ± 21.2457	1972.12 ± 34.973
2.	Poprad, Kišovce-Hôrka	113.13 ± 1.4091	113.13 ± 2.320	18.13 ± 0.2220	18.13 ± 0.365	2338.14 ± 11.6163	2338.14 ± 19.122
3.	Chrámec, Teplá dolina, lokalita 1	181.68 ± 1.1927	181.68 ± 3.149	29.00 ± 0.1911	29.00 ± 0.315	2983.98 ± 5.5217	2983.98 ± 9.089
4.	Chrámec, Teplá dolina, lokalita 2	116.22 ± 1.3164	116.22 ± 2.167	17.78 ± 0.2733	17.78 ± 0.450	1928.53 ± 9.7236	1928.53 ± 16.006
5.	Chrámec, Vlčia dolina, lokalita 3	50.15 ± 1.4464	50.15 ± 2.381	10.26 ± 0.5156	10.26 ± 0.849	1533.66 ± 10.5064	1533.66 ± 17.295
6.	Chrámec, Vlčia dolina, lokalita 4	93.54 ± 1.2831	93.54 ± 2.112	15.08 ± 0.2996	15.08 ± 0.493	1555.83 ± 12.1003	1555.83 ± 19.918
7.	Kráľová, lokalita1	144.90 ± 1.3850	144.90 ± 2.280	14.30 ± 0.1953	14.30 ± 0.322	1619.87 ± 8.0474	1619.87 ± 13.247
8.	Kráľová, lokalita2	122.97 ± 1.4852	122.97 ± 2.455	18.44 ± 0.3855	18.44 ± 0.635	1694.55 ± 17.7395	1694.55 ± 29.201
9.	Priechod-East	100.15 ± 1.3231	100.15 ± 2.178	15.36 ± 0.2104	15.36 ± 0.346	1166.67 ± 12.1799	1166.67 ± 20.050
10.	Priechod-West	113.21 ± 1.5015	113.21 ± 2.472	19.97 ± 0.2200	19.97 ± 0.362	1697.44 ± 11.8001	1697.44 ± 19.424
11.	Priechod-South	107.96 ± 0.8823	107.96 ± 1.452	17.41 ± 0.2290	17.41 ± 0.377	1942.95 ± 4.4873	1942.95 ± 7.387
12.	Lackov	102.70 ± 1.1397	102.70 ± 1.876	16.45 ± 0.4059	16.45 ± 0.668	1617.95 ± 8.4360	1617.95 ± 13.887
13.	Pravica	95.49 ± 1.9416	95.20 ± 3.196	15.06 ± 0.2165	15.06 ± 0.356	1519.55 ± 15.9621	1519.55 ± 26.275
14.	Cerovo	118.02 ± 1.8891	118.02 ± 3.110	20.89 ± 0.1588	20.89 ± 0.261	2030.77 ± 7.2965	2030.77 ± 12.011
15.	Liptovská Lužná	114.08 ± 1.0959	114.08 ± 1.804	14.37 ± 0.3647	14.37 ± 0.60	1508.33 ± 4.2614	1508.33 ± 7.015
16.	Horné lazy	172.07 ± 0.6971	172.07 ± 1.148	26.35 ± 0.2652	26.35 ± 0.437	2426.28 ± 14.4133	2426.28 ± 23.726
17.	Iliaš	166.82 ± 1.1734	166.82 ± 1.932	28.55 ± 0.2628	28.55 ± 0.433	2074.04 ± 12.5205	2074.04 ± 20.610

Legend: * $\bar{x}$—arithmetic mean; s_x—error of the arithmetic mean; **—$\bar{x} \pm$ t. s_x [0.01%]—confidence interval at the probability level of 95 and 99%.

Determination of the antioxidant activity of *J. communis* cone berries collected from different populations in Slovakia by the DPPH and FRAP methods confirmed that the highest antioxidant activity was found in cone berries with the highest content of phenolic substances: Chhrámec Teplá dolina (DPPH: 29.00 ± 0.315%, FRAP: 2983.98 ± 9.089 mgM TE·100 g^{-1}), Horné lazy (DPPH: 26.35 ± 0.437%, FRAP: 2426.28 ± 23.726 mgM TE·100 g^{-1}), and Iliaš (DPPH: 28.55 ± 0.433%, FRAP: 2074.06 ± 2 mgM TE·100 g^{-1}) (Table 5). The lowest antioxidant potential was determined in raw material samples from the following places: Chrámec–Vlčia dolina (DPPH: 10.26 ± 0.849%, FRAP: 1533.66 ± 17.295 mgM TE·100 g^{-1}), Pravica (DPPH: 15.06 ± 0.356%, FRAP: 1519.55 ± 26.275 mgM TE·100 g^{-1}), and Liptovská Lužná (DPPH: 14.37 ± 0.60%, FRAP: 1508.33 ± 7.015 mgM TE.100 g^{-1}).

For the sake of interest, we can mention the determined values of total phenolic content and antioxidant activity for samples of *J. communis* collected from wild populations in Transcarpathia in Ukraine (UA) and near Plovdyv in Bulgaria (BG): Užok—Vihorlat; UA: TPC: 168.62 ± 3.260 mg GAE·100 mL^{-1}, DPPH: 27.30 ± 0.372%, FRAP: 2803.85 ± 5.296 mgM TE·100 g^{-1}; Plovdiv (BG): TPC: 425.98 ± 3.303 mg GAE·100 mL^{-1}, DPPH: 64.21 ± 0.366%, FRAP: 2477.24 ± 28.948 mgM TE·100 g^{-1}.

3.3.1. Cluster Analysis of Juniper Fruit Weight Relationships from Locations and Their Antioxidant Activity

Based on the diagram and table of the distribution of agglomerations (clusters) using the cluster similarity coefficient, we grouped the digital material (the total content of phenols and antioxidant action cone berries of *Juniperus communis* from different places of growth) into five groups (Figure 5).

Case
6. Chrámec, Vlčia dolina, 4
13. Pravica
12. Lackov
15. Liptovská Lužná
9. Priechod-East
7. Kráľová, 1
4. Chrámec, Teplá dolina, 2
11. Priechod-South
1. Spišský hrad, Ostrá hora
8. Kráľová, 2
10. Priechod-West
14. Cerovo
2. Poprad, Kišovce–Hôrka
5. Chrámec, Vlčia dolina, 3
16.Horné lazy
17.Iliaš
3.Chrámec, Teplá dolina, 1

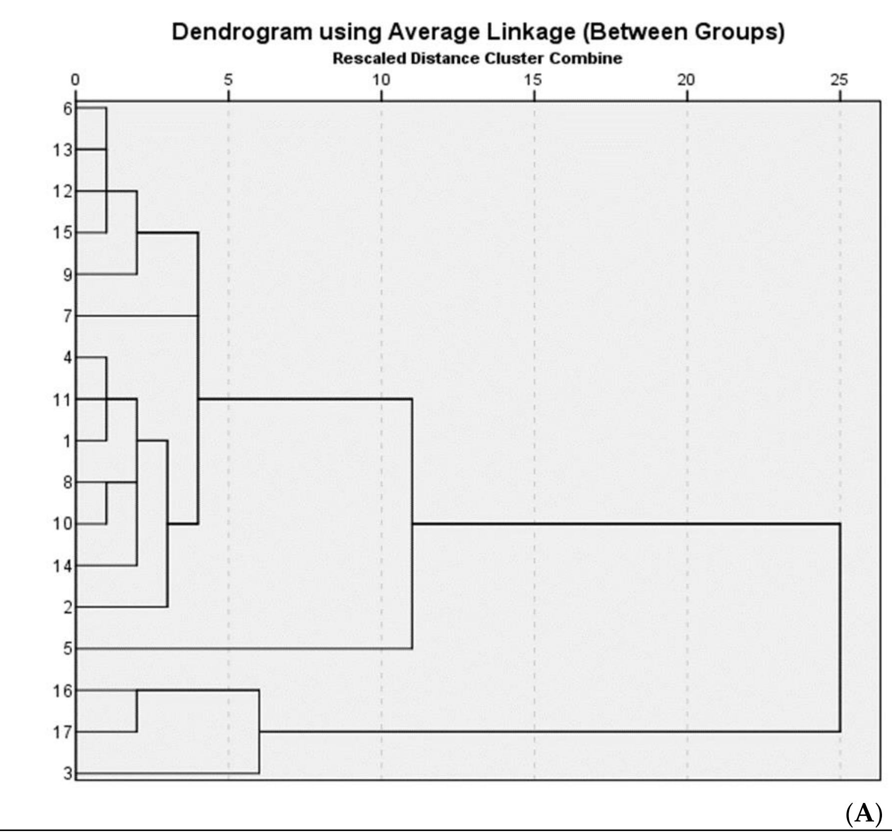


Table of Agglomeration Schedule (**B**)

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	6	13	0.001	0	0	3
2	4	11	0.005	0	0	5
3	6	12	0.013	1	0	6
4	8	10	0.013	0	0	8
5	1	4	0.023	0	2	8
6	6	15	0.023	3	0	9
7	16	17	0.046	0	0	14
8	1	8	0.051	5	4	11
9	6	9	0.052	6	0	12
10	2	14	0.069	0	0	11
11	1	2	0.080	8	10	12
12	1	6	0.133	11	9	13
13	1	7	0.160	12	0	15
14	3	16	0.188	0	7	16
15	1	5	0.387	13	0	16
16	1	3	0.949	15	14	0

Figure 5. Dendrogram of cluster analysis(**A**) and table of distribution of agglomerations (**B**) of *Juniperus communis* populations from different places of growth according to the content of phenols and their antioxidant action.

As can be seen from the cluster analysis (Figure 5) of the distribution of *Juniperus communis* populations according to the content of phenols and the antioxidant action of its cone berries, it can be divided into the following groups:

First group: The group with high phenol content and antioxidant activity (92–100: 91–98:70–81%) in populations from Chrámec: Teplá dolina 1, Horné lazy, and Iliaš.

Second group: In cone berries from the Chrámec place of growth—Teplá dolina 2, Priechod-South, Kráľová 2, Priechod-West, Spišský hrad, Ostrá hora, Poprad, Kišovce–Hôrka, and Cerovo—total phenolic content was average 57–68% and, accordingly, average antioxidant activity was 52–69% and 57–78%.

Third group: Place of growth Chrámec: Vlčia dolina 4, Pravica, Lackov, Liptovská Lužná, and Priechod-East. The content of phenols and the antioxidant activity determined by the DPPH and FRAP methods are low and have a ratio of 51–63:50–57:39–54, respectively.

Fourth group. We isolated the population from Kráľová, lokalita 1, in a separate group. The cone berries contained 80% phenols but low antioxidant action: 49 and 54%.

Fifth group: Chrámec grouping, Vlčia dolina 3 Juniperus cone berries, which had low phenol content and antioxidant activity (28:35:51%).

3.3.2. Dependence of Juniper Fruit Weight and Their Antioxidant Activity on Geographical Distribution

Table 6 and Figure 6 shows the analyzed indicators' dependence on the altitude above sea level and climatic conditions on gentle slopes. As for the cone berries of *J. communis* collected from natural populations in Slovakia, we found three groups according to the TPC, which depended on the location above sea level. The highest content was found in plants from the northern lowlands on gentle slopes of Chrámec, Teplá dolina, locality 1 (181.68 ± 2.007); Horné lazy (172.07 ± 0.732); and Iliaš (166.82 ± 1.231 mg GAE/g extract). Berries of Juniperus cones had the highest antioxidant activity in these populations, from 26 to 29% (DPPH method) and 2983.98:2426.28:2074.04 mgM TE·100 g^{-1} (FRAD method), according to TPC.

The juniper cone berries collected from populations above 400 m above sea level had an average content of TPC and antioxidant activity. TPC (103.6—122.97 mg GAE·100 g^{-1}): 15.04–20.89% (DPPH method): 1928.53–2338.14 mgM TE·100 g^{-1} (FRAP method). Moreover, in cone berries, these indicators were higher on the north, northeast, and northwest side (Kráľová 2, Chrámec, teplá dolina 2, Priechod-West, and Priechod-South) than on the southern side (Poprad, Kišovce–Hôrka, Cerovo, Spišský hrad, and Ostrá hora).

The low content was from the eastern and southern sides on gentle slopes of medium altitudes (Chrámec, Vlčia dolina, lokalita 3—50.15 ± 1.518; Chrámec, Vlčia dolina, lokalita 4—93.54 ± 1.347; Pravica 95.20 ± 2.038). Cone berries from these populations had low antioxidant activity (10.26 (Chrámec, Vlčia dolina 3) to 16.45% (Lackov) and 1166.67 (Priechod-East) to 1617.95 mgM TE·100 g^{-1} (Lackov)). Chrámec grouping, Vlčia dolina 3 Juniperus cone berries had low phenol content and antioxidant activity.

At an altitude of more than 1000 m, the phenolic content was relatively high, but the antioxidant effect was average (Kráľová 2, Kráľová 1).

No correlation was found between the content of phenols, antioxidant activity, and the weight of 100 seeds.

Figure 6 shows the graphic dependence of the antioxidant action on the content of phenols and the weight of 100 seeds. For ease of comparison, the data are converted into percentages of the maximum value of each sample. As can be seen from the diagrams, seed weight is not related to the antioxidant activity of *Juniperus*, while the antioxidant activity is directly proportional to the phenolic content. The higher the content of phenols, the higher the antioxidant action (Chrámec, Teplá dolina, lokalita 1; Horné lazy; Iliaš), located on the northern lowlands on gentle slopes (first group). At the same time, the weight of cone berries from these locations was average. In locations Chrámec, Vlčia dolina, lokalita 4; Chrámec, Vlčia dolina, lokalita 3; and Chrámec, Teplá dolina 2, having the most enormous fruit mass, contained a small number of phenolics and showed the lowest antioxidant activity.

The juniper cone berries collected from populations above 400 m above sea level had an average content of TPC and antioxidant activity. These indicators were greater on the northern side than on the southern side (second group). The cone berries from these populations from the southern and eastern sides on gentle slopes of medium altitudes had low phenol content and antioxidant activity (third group).

At an altitude of more than 1000 m, the phenolic content was relatively high, but the antioxidant effect was average (Kráľová 2, Kráľová 1) (fourth group).

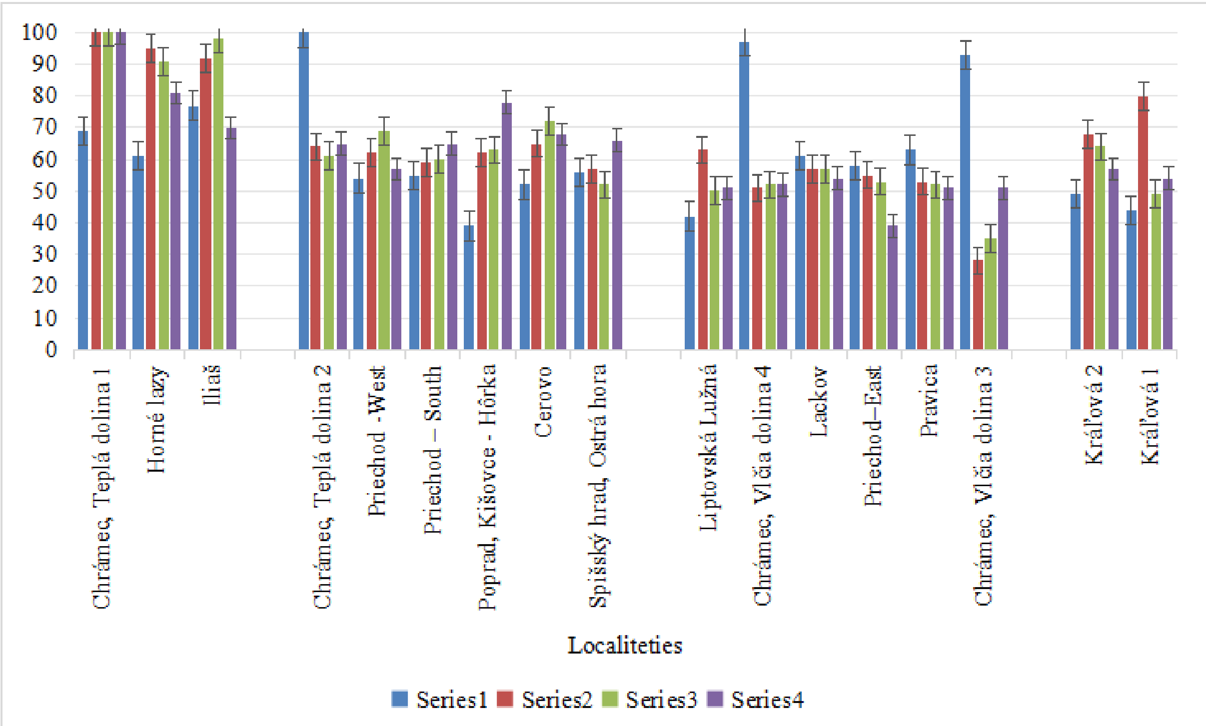


Figure 6. Diagram of the ratio of juniper fruit weight, phenol content, and antioxidant activity in different populations *Juniperus communis* from natural habitats in Slovakia depend on the geographical location: Series 1—weight of 100 seeds (%); Series 2—phenol content (% of the maximum phenol content in the experiment); Series 3—antioxidant activity of *J. communis* cone berries, determined by the DPPH method (%); Series 4—antioxidant activity of *J. communis* cone berries, determined by the FRAP method (%).

Table 6. Phenol content, antioxidant activity, and cone berries' weight depend on the geographical location of *Juniperus communis* populations.

Habitat/ Locality	Weight of Berries (100 Pieces), [g] ($\bar{x} \pm s_X$) *	Total Phenol Content, mg GAE·100 g^{-1} * $\bar{x} \pm t.\ s_X$ * [0.01%]	Antioxidant Activity (DPPH Method), $\bar{x} \pm t.\ s_X$ * [0.01%]	Antioxidant Activity (FRAD Method) mgM TE·100 g^{-1} $\bar{x} \pm t.\ s_X$ * [0.01%]	Geographical Latitude	Terrestrial Longitude	Altitude [m]	Aspect	Slope
1st group—High content of phenols and antioxidant action									
Chrámec, Teplá dolina 1	10.35 ± 0.160	181.68 ± 2.007	29.00 ± 0.201	2983.98± 5.795	N 48°15′35″	E 20°10′56″	248	Northeast	22°
Horné lazy	9.18 ± 0.080	172.07 ± 0.732	26.35 ± 0.278	2426.28 ± 15.126	N 48°48′51″	E 19°36′50″	520	northeast	30°
Iliaš	11.65 ± 0.100	166.82 ± 1.231	28.55 ± 0.276	2074.04 ± 13.140	N 48°41′52″	E 19°18′32″	320	East	16°
2nd group—Average phenolic content and antioxidant action									
Kráľová 2	7.43 ± 0.190	122.97 ± 1.559	18.44 ± 0.405	1694.55 ± 18.616	N 48°52′51″	E 20°08′25″	1010	South	25°
Chrámec, teplá dolina 2	15.08 ± 0.340	116.22 ± 1.381	17.78 ± 0.287	1928.53 ± 10.204	N 48°15′34″	E 20°10′60″	196	South	5°
Priechod-West	8.09 ± 0.170	113.21 ± 1.576	19.97 ± 0.231	1697.44 ± 12.383	N 48°46′45″	E 19°13′54″	480	West	20°
Priechod-South	8.33 ± 0.230	107.96 ± 0.926	17.41 ± 0.240	1942.95 ± 4.709	N 48°46′41″	E 19°13′49″	420	South	15°
Poprad, Kišovce-Hôrka	5.84 ± 0.290	113.13 ± 1.479	18.13 ± 0.233	2338.14 ± 12.191	N 49°02′84″	E 20°38′11″	620	Northeast	30°
Cerovo	7.83 ± 0.260	118.02 ± 1.983	20.89 ± 0.167	2030.77 ± 7.657	N 48°15′17″	E 19°09′26″	468	Northwest	21°
Spišský hrad, Ostrá hora	8.44 ± 0.230	103.60 ± 1.563	15.04 ± 0.303	1972.12 ± 22.296	N 49°00′00″	E 20°46′06″	628	South	30°
3rd group—Low content of phenols and antioxidant action									
Liptovská Lužná	6.27 ± 0.370	114.08 ± 1.150	14.37 ± 0.383	1508.33 ± 4.472	N 48°56′31″	E 19°19′15″	730	Southwest	25°
Chrámec, Vlčia dolina 4	14.64 ± 0.240	93.54 ± 1.347	15.08 ± 0.314	1555.83 ± 12.698	N 48°16′18″	E 20°10′45″	220	South	10°
Pravica	9.43 ± 0.190	95.20 ± 2.038	15.06 ± 0.277	1519.55 ± 16.751	N 48°19′01″	E 19°27′26″	356	Southwest	12°
Lackov	9.24 ± 0.080	102.70 ± 1.196	16.45 ± 0.426	1617.95 ± 8.853	N 48°19′15″	E 19°11′12″	476	Southeast	16°
Priechod-East	8.69 ± 0.200	100.15 ± 1.388	15.36 ± 0.211	1166.67 ± 12.782	N 48°46′40″	E 19°13′50″	390	East	10°
Chrámec, Vlčia dolina 3	14.04 ± 0.270	50.15 ± 1.518	10.26 ± 0.541	1533.66 ± 11.026	N 48°16′24″	E 20°10′54″	250	East	10°
4th group—The high content of phenols and medium antioxidant action									
Kráľová 1	6.59 ± 0.090	144.90 ± 1.453	14.30 ± 0.205	1619.87 ± 8.445	N 48°52′58″	E 20°08′21″	1272	East	35°

Legend: * $\bar{x}$—arithmetic mean; s_x—error of the arithmetic mean; $\bar{x} \pm t.\ s_x$ [0.01%]—confidence interval at the probability level of 95 and 99%.

4. Discussion

The fruits (cone berries) of *J. communis*, in addition to the taste and aroma components, contain fermentable sugars, which in the fermentation process form ethanol, so they are used to produce the alcoholic drink "Borovichka". The alcohol concentration in kvass depends on the fermentation process's total amount of fermentable sugars. Therefore, one needs to monitor Juniperus's sugar content; if it is low, one can sweeten it. The content of aromatic and flavor (especially terpene) components in the distillate depends on their solubility in a specific concentration of spirit. There are also methods in which ethanol is added to the fermented wort to enhance the effect of extractive distillation.

In addition, cone berries contain inverted sugar (30%), glucose + fructose (about 30%), and pectin, as well as malic, ascorbic, and glucuronic organic acids. Lignan contains deoxypodophyllotoxin, cerine, and resins [19]. The results of our research show that the content of reducing carbohydrates in *J. communis* is more significant than in *J. oxycedrus*. Moreover, Albanian *Juniperus* (360 and 197 g/kg) had a 20% higher sugar content than Slovak ones. The composition of carbohydrates included glucose and fructose, the latter of which was approximately 40% less than glucose. Similar studies were obtained when studying the sugar content in juniper cone berries, which grow in the mountains of Turkey at an altitude of 1700 m above sea level. It was established that needles and cones have the highest content of fructose and sucrose, and less glucose and maltose. The content of free sugar in berries exceeds the content of sugar in leaves. Fructose content in cone berries—52.5–69.7 mg/100 g; glucose—35.0–41.7 mg/100 g; sucrose—21.2–29.2 mg/100 g; glucose 10.0–14.6 mg/100 g. The average content of fructose in fresh needles is 44.0–70.2 mg/100 g, sucrose 10.0–25.0 mg/100 g, glucose 22.0–26.0 mg/100 g, and the average content of glucose (maltose) is 5.0–17.0 mg/100 g [20].

When evaluating the content of organic substances in juniper cone berries from different growth places in Slovakia, Bulgaria, and Albania for two years, it was observed that the sugar content in cone berries fluctuated greatly both between populations in one year and different years of collection. The minimum carbohydrate content was 41.6 ± 0.4 g/kg (locality Kišovce), and the maximum was 10 times higher in locality Selčianske sedlo. In contrast, the following year, the carbohydrate content in cone berries from these places was average, and the highest content was in a sample from the Horné lazy locality. Compared to junipers from Albania, all junipers collected from different places in Slovakia have significantly higher carbohydrate content at 300 g/kg. This indicates that the conditions in which juniper cone berries ripen, and their species can influence the quality of the received product. The fruits of *Juniperus drupacea* are used for the production of pekmez (a traditional Turkish fruit concentrate), containing sugar (34.97 g/100 g), ash (3.79 g/100 g), Ca (1499 mg/kg), P (1445 mg/kg), and Zn (12.79 mg/kg) [21].

The content of organic acids ranges from 2.5 ± 0.1 g/kg to 13.3 ± 0.4 g/kg of berries. In most cases, samples of juniper fruits from Slovak locations had values above 10.0 g/kg. The fat content was low and ranged from 8.8 ± 0.18 to 16.18 ± 0.32 percent of the dry matter of juniper cone berries. Moreover, the fat content was somewhat higher in 2017. Also, *J. oxycedrus* has significantly lower organic matter content than *J. communis*. In the following years, dried berries *J. drupacea* collected from two growing areas, which contained total dry matter of 92.89 and 93.30%, water-soluble dry matter of 62.40 and 57.07%, protein content of 2.06 and 3.74%, lipid content of 5.49 and 3.84 g/100 g, pH 5.53 and 5.65, titratable acidity 0.38 and 0.52%, K 14.5 and 17.3 g/kg, Ca 890.5 and 794.7 mg/kg, Na 67.0 and 68.1 mg/kg, Mg 439.2 and 543.6 mg/kg, Fe 33.8 and 65.8 mg/kg, Cu 4.4 and 5.5 mg/kg, Zn 16.5 and 18.1 mg/kg, and, finally, Mn 4.7 and 5.1 mg/kg. In addition, holocellulose (carbohydrates) was determined as 14.29 and 16.01%, lignin (phenolic compounds) as 16.94 and 18.98%, and ash (inorganic components) as 4.00 and 3.38% [22]. Therefore, the lipid content of cone berries from Turkey was significantly lower than in our study. In comparison, junipers from most Slovak populations had the same and significantly higher sugar content.

The results obtained by us can serve as a raw material base for receiving cone berries of *J. communis* for industrial processing, as well as for selecting seed material for industrial cultivation of the species in culture to obtain a more significant quantity of raw materials for industrial processing. Populations at medium altitudes (Priechod-South, Priechod-West, Spišsky hrad, and Selčianske sedlo) are the best for collecting cone berries, which have the highest content of organic substances—fats, carbohydrates, and organic acids. In plants from Král'ová 1, Král'ová 2, Cerovo, Horné lazy, Priechod-East, and Lackov had average fat content. With the ascent to the mountains (Král'ová 1 and Král'ová 2), the content of fats and organic acids in cone berries increased, and sugars decreased. In juicy fruits, such as juniper, the pericarp consists of three parts: exocarp, mesocarp, and endocarp. The exocarp is leathery, externally covered with a thick layer of wax coating cuticle protecting the fleshy internal contents (mesocarp and endocarp) and seeds from drying out and freezing. This is why plants growing above 700 m above sea level have a much higher fat content.

In the mountains of Turkey at an altitude of 1700 m, the fructose content in berries from the north side is higher than from the south [19]. The content of organic substances was weakly correlated with the weight of 100 fruits from natural populations of *J. communis* in Slovakia. Large fruits were collected from plants on the lower, northern slopes of the mountains (10–15 g weight of 100 pieces of juniper fruit), but they had an average content of organic substances. Meanwhile, medium-sized cone berries were harvested at medium altitudes and had relatively high contents of all organic investigated substances, and sometimes, they had medium fat content. Cone berries collected higher than 700 m above sea level had tiny seeds and low organic substance contents.

Italian scientists found that the sugar content is affected by the time of picking cone berries. So, the highest sucrose concentration was in spring (15.47 mg/g); it decreased in summer to 1.10 and in autumn to 4.75 and disappeared in winter, whereas a-glucose, b-glucose, and fructose are practically absent during spring, summer, and autumn, and the maximum concentrations were in winter (52.37; 26.62; 54.82 mg/g, respectively), when sucrose disappears. The high sucrose content in the spring and its significant decrease in other periods can be explained by low or absent enzymatic activity during the growing season, when glucose is needed as an energy source. In contrast, fructose and glucose accumulate in the winter during the dormant period, when they act as storage compounds [23].

Dried bluish-black juniper cone berries are used as flavoring in food products and alcoholic beverages; their extracts are widely used in medicine for their antimicrobial, antifungal, and anticancer effects. In juniper cone berries, 26 metabolites were identified, including sugars, amino acids, organic acids, and triterpenes [23]. Organic substances, particularly sugars and essential oils, are important for the taste qualities of an alcoholic drink, "Spisska Borovichka". The most valuable are chemotypes of essential oils with high a-pinene content. It was experimentally shown that a-pinene has the best pharmacological effects—anti-inflammatory, antispasmodic, antitumor, gastroprotective, and antibacterial [24,25].

Our research has shown that *J. communis* cone berries exhibit strong antioxidant properties. Antioxidants are substances that fight the adverse effects of oxygen-induced oxidation processes in the organism. Oxidative stress is a process in the organism when the formation of harmful molecules, free radicals, prevails because the organism does not have enough antioxidants to neutralize these molecules. Free radicals bind to compounds in the cell, disrupting the ordinary course of the metabolic process or preserving hereditary information. The high TPC values recorded in all alcoholic extracts of Juniperus mean that beverages and natural supplements made from this raw material can bind free radicals and have antioxidant action.

In juniper cone berries, the purple and blue color is due to anthocyanins, mainly flavonoids, which provide antioxidant action, improve our vision, have anti-inflammatory action, and have a good influence on blood vessels. Several professional works have shown that cone berries and their ethanolic and ethyl acetate extract of both standard Juniperus and red Juniperus have potent antioxidant activity. A significant correlation was also demonstrated between the content of phenolic substances and flavonoids regarding antioxidant potential for all the investigated extracts.

In addition to essential oils, several other compounds were found in the cone berries of *J. communis*. Diterpenes—sugiol, xanthoperol, 4-epi-abietic acid, 4-epi-dehydroabietic acid, 4-epi-palustric acid, 4-epi-abietinal, 4-epiabietinol, isopimaric acid, isocommunic acid, [-] ent-trans communic acid, and sandracopimaric acid. Neolignan glycosides—junipercomnoside A and B and icariside E4. Juniper berries also contained lignans—podophyllotoxin, tannins, and gallocatechins—and flavonoids [scutellarein, luteolin-7-O-b-D-glucoside, nicotiflorin, kaempferol-3-O-β-D-glucoside, Kaempferol-3-O-a-rhamnopyranoside, Quercetin-3-a-O-L-rhamnopyranoside, Quercitrin, Isoquercitrin, Quercetin-3-O-arabinosyl-glucoside, rutin, quercetin, luteolin, apigenin, amentoflavone, isocutellarein, hypolaetin, kaempferol 3-O-alpha-rhamnopyranoside, nicotiflorin and naringenin] [6]. Thus, 15 phenolic compounds were found in *Juniperus communis* cone berries. Their main groups were flavones, flavonols, phenolic acids, flavanol, and biflavonoids, including quercetin glycosides, apigenin, isoscutellarein, and hypolaetin [26]. Several researchers associate antioxidant activity with the presence of phenols in *Juniperus* cone berries, which was also confirmed by our research. The higher the phenol content, the higher the antioxidant capacity. High phenol content was found in the industrial sample of *J. communis* from Serbia and Albania (417.21 and 365.91 mg GAE·100 g^{-1}, respectively), which is related to the cone berries being sorted for sale. Thus, the content of phenols and antioxidant capacity in the industrial sample of *J. communis* from Serbia was the highest and amounted to 100%, that from Albania being 12–20% less. These plants had the highest antioxidant activity (63.37 and 51.04% by the DPPH method, and 29,500 and 2408.7 mgM TE·100 g^{-1} by the FRAP method). In other investigated species—*J. oxycedrus* from Albania and *J. communis* from the Czech Republic and Bosnia—the content of phenols and antioxidant activity was 40–50% lower.

The contents of phytochemicals, total phenolics, total flavonoids, and antioxidant potential of extracts of *Juniperus communis* and *J. oxycedrus* berries were determined. Ethanol, ethyl acetate, and chloroform were used for extraction; antioxidant activities were determined by DPPH assay. Ethanolic extract of *Juniperus communis* L. possesses the most potent antioxidant activity (IC50 = 28.55 ± 0.24 µ/mL), as well the higher contents of total phenolics, 189.82 ± 0.27 mg of gallic acid equivalent per g of dried weight extract (mg GAE/g extract DW), and total flavonoids, 42.85 ± 0.13 mg of rutin equivalents per g of dried weight extract (mg RE/g extract DW). Ethanolic extract of *J. oxycedrus* had higher antioxidant activity (IC50 = 64.49 ± 0.23 µ/mL), lower contents of total phenolics, 58.73 ± 0.14 (mg GAE/g DW), and total flavonoids—21.39 ± 0.33 (mg RE/g extract DW). The results indicated the potential application of the tested extracts as significant antioxidants [27].

As for the cone berries of *J. communis* collected from natural populations in Slovakia, we found three groups according to the TPC, which depended on the location above sea level. The highest content was found in plants from the northern lowlands of Chhrámec, Teplá dolina, lokalita 1 (181.68 ± 2.007); Horné lazy (172.07 ± 0.732); and Iliaš (166.82 ± 1.231 mg GAE/g extract). The lowest content was from the eastern and southern sides on gentle slopes of medium altitudes (Chrámec, Vlčia dolina, lokalita 3—50.15 ± 1.518; Chrámec, Vlčia dolina, lokalita 4—93.54 ± 1.347; Pravica 95.20 ± 2.038).

When analyzing the content of phenols and their antioxidant action, it turned out that the best in terms of content are populations located on the northern, rather steep slopes in the lowlands of the Slovak Carpathians. These indicators fell with the rise in the mountains, especially from the southern side. At the same time, no correlation was found between the content of phenols, antioxidant activity, and the weight of 100 seeds.

The literature shows that the antioxidant action is directly proportional to the content of phenols [27]. When studying the total content of polyphenols (by the Folin–Ciocalteu method) of methanolic extracts of cone berries of two subspecies of *Juniperus communis* L. var. *communis* (Jcc) and *Juniperus communis* L. var. *Saxatilis* Pall. (Jcs) from Turkey, it was found that the total polyphenol content was three times higher in Jcc (59.17 ± 1.65 mg GAE/g extract) than in Jcs (17.64 ± 0.09 mg GAE/g extract). In this study, the content of TPC was significantly lower (by between three and two times) than what we determined in the cone berries collected from Slovakia and six times lower than from the commercial network of Serbia and Albania (417.27 and 365.91 mg GAE·100 g^{-1}). The content of flavonoids and biflavonoids, evaluated by HPLC-DAD-ESI-MS analysis, was five times higher in Jcc (25,947 ± 0.86 and 4346 ± 3.95 µg/g extract) than in Jcs (5387 ± 34.88 and 1944 ± 26.88 µg/g extract) [28]. HPLC analysis allowed for isolating 16 flavonoids, including hypolaethin-7-pentoside, quercetin–hexoside, gossypetin–hexoside–pentoside, and gossypetin–hexoside. In Jcs, eight flavonoids were identified; quercetin–hexoside and isoscutellarein-8-O-hexoside are the most abundant compounds. Accordingly, the antioxidant activity of Jcc is greater than that of Jcs in the DPPH test (IC (50) 0.63 ± 0.09 mg/mL and 1.84 ± 0.10 mg/mL) in the reducing power test (12.82 ± 0.10 ASE/mL and 64.14 ± 1.20 ASE/mL) and in the TBA test (IC 50 4.44 ± 0.70 µg/mL and 120.07 ± 3.60 µg/mL). On the contrary, Jcs showed a greater Fe^{2+} chelating capacity than Jcc. Extracts of both subspecies only had antimicrobial activity against Gram-positive bacteria [28].

The phenolic-enriched ethyl acetate fraction of the extract of *J. communis* leaves exhibited potent hepatoprotective activity. The hepatoprotective potential may result from their antioxidant potential against active forms of oxygen and nitrogen, which prevent lipid peroxidation, ultimately preventing the necrosis or apoptosis of liver cells. The leaves of *J. communis* can be included in nutraceutical preparations with marked benefits for human or animal health [29].

In other research, aqueous and ethanolic extracts with total phenolic content of 16.58 and 16.20 mg/g demonstrated solid antioxidant activity. Concentrations of 20, 40, and 60 µg/mL aqueous and ethanolic extracts of *Juniperus communis* fruits showed 75%, 88%, 93%, 73%, 84%, and 92% inhibition of linoleic acid emulsion peroxidation, respectively. Both Juniperus extracts had effective restorative action, free radical absorption, superoxide radical anion absorption, hydrogen peroxide absorption, and metal chelation at the same concentrations [20, 40, and 60 µg/mL] [30].

Plant phenolic compounds have been shown to activate apoptotic cell death in various oncogenic cell lines. Juniperus berry extract activated p53 cellular re-localization and DNA fragmentation-dependent death of SH-SY5Y human neuroblastoma cells [26].

Based on the cluster analysis of the content of organic substances in juniper cone berries and their antioxidant activity, collected from different populations of Slovakia, we selected three groups. When comparing them, several regularities were revealed.

The juniper cone berries growing in the Carpathian lowlands had an average content of organic substances: fats—10.2 ± 0.4–11.9 ± 0.24 g/kg in DW; sugars—315.9 ± 3.2–367.5 ± 17.00 g/kg; and organic acids—8.0 ± 0.3–12.7 ± 0.44 g/kg in DW. The plants from the best in terms of content of phenols and antioxidant activity are the populations located on the northern, rather steep slopes, in the lowlands of the Slovak Carpathian of Chhrámec, Teplá dolina, locality 1; Horné lazy; and Iliaš (181.68 ± 2.007 mg GAE·100 g^{-1}: 29.00 ± 0.201, % (DPPH method): 2983.98 ± 5.795 mgM TE·100 g^{-1} (FRAP method).

The best content of organic substances was found in plants at medium altitudes (Priechod-South, Priechod-West, Spišsky hrad, Selčianske sedlo, Cerovo, Horné lazy, Priechod-East, and Lackov): fats—8.5 ± 0.3–15.3 ± 0.6 g/kg; sugars—265.7 ± 2.7–416.7 ± 4.2 g/kg; and organic acids—8.6 ± 0.3–12.7 ± 0.4 g/kg in DW. Plants from these populations can be the raw material of cone berries for the food industry. Moreover, on the northern side, the indicators of the content of organic substances were lower than on the southern side.

On the other hand, cone berries from these populations had average phenolic content and antioxidant activity. Cone berries from populations located above 400 m above sea level from the north, northeast, and northwest sides had average TPC and antioxidant activity (Poprad, Kišovce–Hôrka, Cerovo, Spišský hrad, Ostrá hora, Chrámec, teplá dolina 2, Priechod-West, Priechod-South): TPC (103.6–122.97 mg GAE·100 g^{-1}); 15.04–20.89% (DPPH method); 1928.53–2338.14 mgM TE·100 g^{-1} (FRAD method). The lowest content came from the eastern and southern sides on gentle slopes of medium altitudes (Liptovská Lužná, Chrámec, Vlčia dolina 4, Pravica, Lackov, Priechod-East, Chrámec, Vlčia dolina 3):

TPC (50.15 ± 1.518–114.08 ± 1.150 mg GAE·100 g^{-1}); 10.26 ± 0.541–15.06 ± 0.277% (DPPH method); 1166.67 ± 12.782–1555.83 ± 12.698 mgM TE·100 g^{-1} (FRAD method).

Juniper cone berries from plants growing above 700 m above sea level had increased fat and organic carbohydrate content. However, they decreased sugar content (fats—10.4 ± 0.4–15.3 ± 0.6 g/kg; sugars—41.6 ± 0.4–270.9 ± 2.7 g/kg; and organic acids—7.7 ± 0.2–10.3 ± 0.3 g/kg in DW). At an altitude of more than 1000 m, the phenolic content was relatively high, but the antioxidant effect was average (Kráľová 2, Kráľová 1) (144.90 ± 1.453: 14.30 ± 0.205: 1619.874 ± 8.445, in accordance).

Based on the cluster analysis of *Juniperus* populations growing in Slovakia according to the total content of phenols and the antioxidant action of its cone berries, Juniperus places of growth can be divided into four groups. Comparing these groups with the groups separated by essential oil content, the investigation carried out earlier found the following regularity: the higher the content of phenols and essential oils dominated by α-pinene, the higher the antioxidant action. There are exceptions, possibly related to climatic features in the year of research [31]. Therefore, our research suggests using *J. communis* berries in the Slovak national drink "Borovichka" production due to the high content of α-pinene, which is the donor of the necessary aroma.

The content of juniper cone berries of primary and secondary synthesis substances depends on their origin and ripening period. In Slovakia, the cone berries of *J. communis* are the primary raw material used to produce the historically traditional Slovak alcoholic drink "Spisska borovichka". Today, cone berries are mainly imported from Albania, where the plants usually grow on rocky, infertile areas, fields, meadows, forest clearings, sparse forests, etc. There is a need to find new natural populations or to develop a technology for growing *J. communis* in culture to meet the needs of the pharmaceutical and food industries.

The results obtained by us can serve as a raw material base for receiving cone berries of *J. communis* for industrial processing, as well as for selecting seed material for industrial cultivation of the species in culture to obtain a more significant amount of raw materials for industrial processing. These are the populations of Chrámec, Teplá dolina, 1; Horné lazy; Iliaš; as well as cone berries collected from places of growth Chhrámec, Teplá dolina 2; Priechod-South; Kráľová 2; Priechod-West; Spišský hrad, Ostrá hora; Poprad, Kišovce—Hôrka; Cerovo; and Kráľová, lokalita 1, which had relatively large cone berries with medium to high content of carbohydrates; essential oils, half of which comprised α-pinene; phenols; and medium to high antioxidant activity.

5. Conclusions

Based on a cluster analysis of the content of organic substances in juniper cone berries and their antioxidant activity, collected from different populations of Slovakia, we selected four groups. When comparing them, several regularities were revealed.

Juniper cone berries growing in the Carpathian lowlands had an average content of organic substances. The plants from the northern lowlands of Chhrámec, Teplá dolina, locality 1, Horné lazy, and Iliaš had the highest content of phenol and antioxidant activity.

The best content of organic substances was found in plants at medium altitudes (Priechod-South, Priechod-West, Spišsky hrad, Selčianske sedlo, Cerovo, Horné Lazy, Priechod-East, and Lackov). Moreover, on the northern side, the indicators of the content of organic substances were lower than on the southern side.

On the other hand, cone berries from these populations had average phenolic content and antioxidant activity. Cone berries from populations located above 400 m above sea level from the north, northeast, and northwest sides had an average content of TPC and antioxidant activity (Poprad, Kišovce–Hôrka, Cerovo, Spišský hrad, Ostrá hora, Chrámec, teplá dolina 2, Priechod-West, and Priechod-South). The low content was from the eastern and southern sides on gentle slopes of medium altitudes (Liptovská Lužná, Chrámec, Vlčia dolina 4, Pravica, Lackov, Priechod-East, Chrámec, Vlčia dolina 3).

Juniper cone berries from plants growing above 700 m above sea level had increased fat and organic carbohydrate content but decreased sugar content. At an altitude of more than 1000 m, the phenolic content was relatively high, but the antioxidant effect was average (Kráľová 2, Kráľová 1).

So, based on the conducted research, it was established that the content of organic substances, particularly sugars, in juniper cone berries decreased with elevation in the mountains and was the best at medium altitudes. The best places to collect raw materials are populations from middle latitudes. At the same time, the antioxidant effect was better in cone berries collected at all altitudes, but only from the north, northeast, and northwest sides. Populations from areas of growth in Chhrámec, Teplá dolina, lokalita 1, Horné lazy and Iliaš, Priechod-South, Priechod-West, Spišsky hrad, Selčianske sedlo, Cerovo, Horné Lazy, Poprad, Kišovce–Hôrka, Spišský hrad, Ostrá hora, Chhrámec, teplá dolina 2, Priechod-West, Priechod-South. Kráľová 2 and Kráľová 1 present the best raw material base for the production of the Slovak national alcoholic drink "Borovichka".

Author Contributions: M.H. processed the experimental data, performed the biochemical–biological analysis, and drafted the manuscript. I.S. carried out almost all the technical details and performed numerical calculations for the suggested experiment with plant population collection in Slovakia. R.P. devised the project of juniper biodiversity, the main conceptual ideas, and the proof outline. V.V. carried out DPPH and FRAP analyses and collected the juniper fruits in various habitats in Slovakia. All authors have read and agreed to the published version of the manuscript.

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