

Phytochemical Profile of Ripe *Juniperus excelsa* M. Bieb. Galbuli from Bulgaria

S. Stankov, H. Fidan, N. Petkova, M. Stoyanova, Tz. Radoukova, A. Stoyanova

Abstract—The aim of this study was to evaluate the chemical composition of ripe *Juniperus excelsa* M. Bieb. galbuli (female cones) collected from "Izgoraloto Gyune" Reserve in Krichim, Bulgaria. The moisture (36.88%), abs. weight 693.96 g/1000 pcs., and the ash content (10.57%) of ripe galbuli were determined. Lipid fraction (9.12%), cellulose (13.54%), protein (13.64%), and total carbohydrates (31.20%) were evaluated in the ripe galbuli. It was found that the ripe galbuli contained glucose (4.00%) and fructose (4.25%), but disaccharide sucrose was not identified. The main macro elements presented in the sample were K (8390.00 mg/kg), Ca (4596.00 g/kg), Mg (837.72 mg/kg), followed by Na (7.69 mg/kg); while the detected microelements consisted of Zn (8.51 mg/kg), Cu (4.66 mg/kg), Mn (3.65 mg/kg), Fe (3.26 mg/kg), Cr (3.00 mg/kg), Cd (< 0.1 mg/kg), and Pb (0.01 mg/kg).

Keywords—Chemical composition, *Juniperus excelsa* M. Bieb. minerals, ripe galbuli.

I. INTRODUCTION

JUNIPERS (*Juniperus*) are a genus of conifers and shrubs of the Cupressaceae family. Depending on the taxonomic criteria, 50 to 67 species are recognized and widespread in the northern hemisphere (from the Arctic in the south to tropical Africa, as well as in the mountainous parts of Central America) [1].

There are five species in Bulgaria: common (blue) juniper (*J. communis* L.), red juniper (*J. oxycedrus* L.), low juniper (*J. pygmaea* C. Koch), fragrant juniper (*J. sabina* L.) and juniper (*J. excelsa* M. Bieb.) as the latter two were being rare and protected.

The Bulgarian juniper leaves are an industrial raw material for the production of essential oil and juniper water, which are used in the perfume and cosmetics industry [2]. Over the years, mainly the chemical composition of the essential oil of leaves and fruits of individual species growing in the region, for example, *J. communis* [3]-[7], *J. excelsa* [8]-[10] and other species [11], [12] was studied.

Studies on the chemical composition and the essential oil properties of juniper species growing in different countries around the world, for example by *J. communis* [13], by *J. excelsa* [14], [15], of other species [16] have been conducted by a number of authors.

Stankov, S., Petkova, N., Stoyanova, M., and Stoyanova, A. are with the University of Food Technologies, Plovdiv, Bulgaria (e-mail: docstankov@gmail.com, petkovanadejda@abv.bg, magdalena.stoianova@abv.bg, aastst@abv.bg).

Fidan, H. is with the University of Food Technologies, Plovdiv, Bulgaria (corresponding author, phone: +359896723605; e-mail: hfidan@abv.bg).

Radoukova, Tz. is with the Paisii Hilendarski University of Plovdiv, Bulgaria (e-mail: radukova@uni-plovdiv.bg).

The galbuli of the different species of the genus *Juniperus* can be described as conical grains, in the internal structure of which are found fleshy almost fused scales. Some of them, such as *J. communis* [13], [17], *J. drupacea* [18], *J. phoenicea* [19], [20], *J. depeana* [21], *J. oxycedrus* [22] and *J. excelsa* [23] are processed and used in the food and pharmaceutical industries. Others, such as *J. sabina* fruits, are a source of toxic substances, and their consumption is not recommended [24].

Nowadays, science worldwide is turning its attention to alternative sources of micro and macronutrients as part of a rational human diet [25]. Many plant materials are a major source of bioactive compounds most commonly related to phenolic substances, proteins, lipids, and carbohydrates [26].

In combination with each other, they promote hormonal balance and actively participate in the metabolic processes of a living organism [27].

The inclusion of a variety of food sources improves the body's nutritional balance with water, carbohydrates, organic acids, dyes, vitamins, polyphenols and others [28], and on the other hand increases the quality of food in terms of texture, color, aroma and taste. Some Eastern countries use the fruits of the species *J. communis* in the composition of traditional dishes, pre-dried and used in the form of spices [29]. Today, most of the species of the genus *Juniperus* are protected plant species, which is why they are less studied for industrial processing.

There are relatively few data on the phytochemical composition of ripe galbuli of *J. excelsa*. The lack of generalized data on the composition and properties of plant species creates some limits in their use. For this reason, the aim of the present study was to present a generalized phytochemical composition of the ripe galbuli of *J. excelsa* as a source of biocomponents.

II. MATERIALS AND METHODS

A. Materials

Plant material (galbuli, berries, fruits) of *Juniperus excelsa* M. Bieb. (branches not thicker than 10 mm) was collected on January, 2020 from the reserve "Izgoraloto Gyune", above the town of Krichim, Bulgaria (42°01'40"N, 24°28'09"E, 367 masl).

The galbuli were placed in paper sacks and stored in a cool, dry, well-ventilated, and dark cabinet (Fig. 1). Before analysis, the galbuli were finely ground with a laboratory mill (Clatronic KSW 3307 Grinder). The biologically active substances in the samples were analyzed and the values were represented based on the absolute dry weight.



Fig. 1 Ripe *J. excelsa galbuli* from (authors' image)

B. Methods

Absolute Weight

The absolute weight of the sample was determined for 1000 randomly selected seeds (air-dried) using an electronic precision balance (Mettler-Toledo, ± 0.0001 g).

Moisture

The initial moisture content of the sample was determined by drying at 103 ± 2 °C to constant weight, and all results were given on a dry weight (dw) basis [30].

Ash

The ash content of the sample was determined by heating at 600 °C for 5 h.

Lipid Fraction

The lipid fraction was extracted from the ground sample using hexane in a Soxhlet apparatus for 8 h. The solvent was partially removed in a rotary vacuum evaporator, the residue was transferred in a pre-weighed glass vessel and the rest of the solvent was removed under a stream of nitrogen to a constant weight to determine the oil content [31].

Crude Cellulose

The finely ground sample (0.5 g to 1 g) was placed in a round bottom flask of 250 mL and 16.5 mL acid mixture containing 80% acetic acid and nitric acid was added (450 mL 80% acetic acid were mixed with 45 mL concentrated nitric acid). The flask with a sample was heated at boiling temperature for 30 min with periodically shaking. Then the sample was filtered through previously tarred sintered glass (G2). The sample was washed with the hot acid mixture, then with hot water, and 95% ethanol to remove the residual acids. After that 10 mL diethyl ether were added to the residue on the sintered glass filter and the washing process was repeated with hot water, 95% ethanol, and diethyl ether. At the end the sintered glass with residual sample was dried to the constant weight at in an oven at 105 °C, cooled down in desiccator and then weighted [32].

Total Carbohydrates

The total soluble carbohydrate content was evaluated by phenol-sulphuric acid method. In brief, 100 μ l properly diluted hydrolyzed extract was mixed with 1 mL 5% phenol and 5 mL concentrated sulphuric acid. The samples were placed in a water bath at 30 °C for 20 min and then the absorbance was measured at 490 nm against blank with d. H₂O [33]. The amount of carbohydrates was determined from the calibration curve with glucose. The results were calculated as a percent (%) of dry weight (dw).

HPLC-RID Analysis of Sugars

The sample was extracted with distilled water in solid to liquid ratio 1:5 (w/v) in an ultrasonic bath (VWR, Malaysia) with frequency 45 kHz and 30 W power at 45 °C. The ultrasound-assisted extraction was performed in triplicate. Samples were extracted. The sugar analysis of water extracts was performed on an HPLC instrument Elite Chrome Hitachi with a refractive index detector (RID) Chromaster 5450 on a Shodex[®] Sugar SP0810 (300 mm \times 8.0 mm) with Pb²⁺ and a guard column Shodex SP - G (5 μ m, 6 \times 50 mm), operating at 85 °C, with a mobile phase distilled H₂O and a flow rate of 1.0 ml/min [34].

Macro- and Micro-Minerals

Air-dried sample was mineralized at 450 °C. The residue was first dissolved in concentrated HCl and evaporated to dryness, then the remainder was dissolved in 0.1 mol.L⁻¹ HNO₃ solution. Mineral contents were determined on an atomic absorption spectrophotometer (AAS) Perkin Elmer/HGA 500 (Norwalk, USA), under the following instrumental parameters for the flame AAS: sodium (Na) 589.6 nm; potassium (K), 766.5 nm; magnesium (Mg), 285.2 nm; calcium (Ca), 317.0 nm; zinc (Zn), 213.9 nm; copper (Cu), 324.7 nm; iron (Fe), 238.3 nm; and manganese (Mn), 257.6 nm. Identification of metals was carried out by comparison to a standard solution of metal salts, and metal concentrations were calculated from a calibration curve, built by using a standard 1 μ g.mL⁻¹ solution.

III. DISCUSSION

The ripe *J. excelsa galbuli* had abs. weight 693.96 g/1000 pcs. There was no data on this indicator for the same species in the literature. The reported value was significantly higher than the data for *J. communis* (117.2 g/1000 pcs), which may be due to the differences in humidity (7.50-9.00%) and species specificity [35].

The results for the micro and macronutrients were presented in Fig. 2.

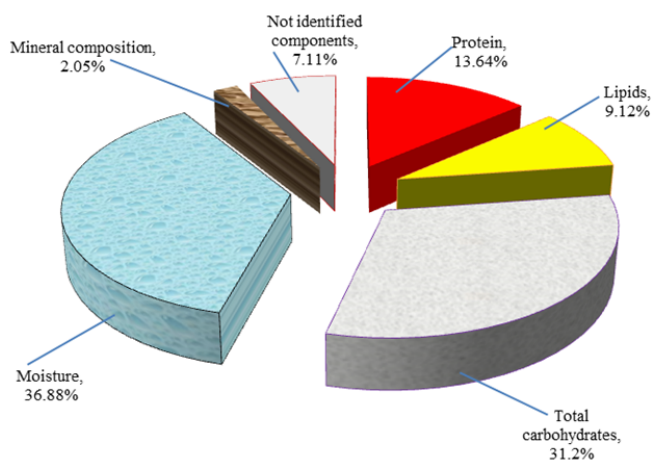


Fig. 2 Phytochemical composition of ripe *J. excelsa galbuli*

The moisture content for the *J. excelsa galbuli* (36.83%)

was higher than the moisture content of *J. drupacea* fruits from Turkey (32.15%), which was a consequence of the harvest period and the climatic conditions under which the fruits grow [16]. The four main groups of components have been identified in the composition of *J. excelsa* galbuli - lipid fraction, proteins, cellulose, and carbohydrates.

Carbohydrates had the largest share of the nutrients, as the share of cellulose was 13.54%, and glucose (4.00%) and fructose (4.25%) were comparable with the levels of sugars in ripe fruits of *J. communis* (4.30-5.00%) previously reported in Bulgaria [35]. The data on total carbohydrates were comparable to those obtained by other researchers [36], [37], in which most of the total carbohydrates were lignocellulosic substances. Türkoglu et al. [38] defined the different levels of reducing sugars with the degree of fruit ripeness and climatic conditions of the genus *Juniperus*. The high values of the lignocellulose components confirm the woody character of the fruits, which makes them unsuitable for direct consumption. The presence of a high percentage of carbohydrates, including lignin fractions, is a prerequisite for a high content of phenolic compounds [39] which can be used as a valuable biocomponent as well as the possibility to be included in the composition of flavoring and marinating composite mixtures in the processing of meat products or teas.

The results presented in Fig. 2 show that the protein content was higher than the amount obtained in the *J. drupacea* (2.06-3.74%), found by [16]. Protein content was 4% in the composition of ripe fruits of the species *J. occidentalis* [37]. In [17] the potential possibilities for the use of *J. communis* fruits in the form of compound feed for birds, in which high yields of muscle mass, were reported.

The low levels of the lipid fraction in the ripe galbuli were comparable to the data for other juniper species, as *J. occidentalis* - 16% [37] and *J. phoenicea* - 11% [19]. For the *J. drupacea* species, it is stated that the lipid fraction varied depending on the altitude and age of the trees, as at lower altitude the lipid fraction was 5.49%, and at higher (over 1,200 m) it is 3.84% [16].

The determined ash content (10.57%) was higher than that of ripe fruits of the species *J. drupacea* (3.69%) [16] and *J. communis* – among (3.77%) [40] and (2.90%) [35]. This was explained by the higher levels of total carbohydrates contained in the test sample.

Data on the composition of the identified mineral elements are presented in Table I. The results show high values of macronutrients in the composition of ripe fruits. The relatively diverse composition of micro and macroelements is due to differences in climatic conditions and species diversity [41], [42].

The mineral composition was comparable with the data from [16] for fruits of the species *J. drupacea*. The results obtained in this study were higher than those reported by [16] for Ca content (842.6 mg/kg), followed by Mg (491.4 mg/kg), and Mn (4.9 mg/kg).

Inci et al. [17] identified Fe (80.12%), Cu (19.94%), and Mn (15.31%) as the main elements in the mineral composition of *J. communis* fruits. In another study, [42] reported that Ca

was with the highest values (1052-7260 mg/kg) in fruit samples collected in different locations, altitude, groundwater level, and acidity of the soil. According to the authors, the main reason for obtaining quantitative and qualitative differences in the composition of minerals is the soil and climatic conditions in which the fruit grows. In ripe fruits of *J. phoenicea*, [19] identified Na (6.38 mg/100 g) and K (373.9 mg/100 g), which is several times lower than that of *J. excelsa* galbuli. The amount of Cr in *J. excelsa* galbuli (3.00 mg/kg) is relatively high, which could make it suitable for including in animal feed production, as chromium together with some proteins is involved in the regulation of glucose in blood flow and supports the development of the muscular and skeletal system [43].

TABLE I
 MACRO- AND MICRO-ELEMENTS CONCENTRATIONS IN RIPE *J. EXSELSA*
 GALBULI

Macro-elements	Content, mg/kg	Micro-elements	Content, mg/kg
Potassium (K)	8390.00	Zinc (Zn)	8.51
Calcium (Ca)	4596.00	Copper (Cu)	4.66
Magnesium (Mg)	837.72	Manganese (Mn)	3.65
Sodium (Na)	7.69	Iron (Fe)	3.26
		Chromium (Cr)	3.00
		Cadmium (Cd)	< 0.1
		Lead (Pb)	0.01

The amount of Zn (8.51 mg/kg) was higher or close to its content in foods such as garlic (*Allium sativum*), celery (*Apium graveolens*), cabbage (*Brassica oleracea*), broccoli (*B. var. Italica*), carrot (*Daucus carota*), lettuce (*Lactuca sativum*), pea (*Pisum sativum*) and potato (*Solanum tuberosum*) [44].

The balanced nutritional composition of *J. excelsa* galbuli makes it a potential source of nutrients that can be included in a number of foods.

IV. CONCLUSIONS

The present study summarized data on the nutritional composition of ripe *J. excelsa* galbuli as an unconventional source of nutrients and bioactive compounds. The inclusion of *J. excelsa* galbuli in the composition of model food systems and the study of their impact on the vital indicators of living organisms would expand the database with potential possibilities for their usage.

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