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SOME FEATURES OF THE SPECIES OF THE GENUS JUGLANS L IN THE CONDITIONS OF BRYANSK OBLAST

Abstract: The genus *Juglans*, family *Juglandaceae*, comprises several species. It is widely distributed throughout the world. Green walnuts, shells, seeds, barks, and leaves are used in the pharmaceutical and cosmetic industries. Leaves are easily available in abundant amounts. The analysis was conducted for several purposes. Firstly, the elemental compositions of various parts of the leaves and immature fruits of four species of the genus *Juglans L*, growing in the conditions of Bryansk region was determined. Ten elements were detected by using electron-ion raster scanning microscope «Quanta 200 3D» in nanocenter NIU "BSU" and percentages of macronutrients and micronutrients in the leaves and immature fruits were determined. The analysis was also conducted to determine quantification of various substances: flavonoids, organic acids, vitamin C, antioxidant activity, dry matter, chlorophyll, carotenoid, free and bound water. This data can be used in the pharmaceutical industry. Secondly, the morphology and anatomy of the leaf and water-holding capacity of four species of the genus *Juglans L* were also identified. Therefore, this result can prove the difference among the species of the genus *Juglans L*.

Key words: Anatomy, species of the genus *Juglans L*, leaf, chlorophyll, immature fruit, chemical composition.

Language: English

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Introduction

It is well known that the species of the genus *Juglans L* are valuable fruit trees example [6, p.95-98; 15]. They have high economic value. They are widely used in medical and pharmaceutical industries. The fruits contain up to 76% fat, 22% protein and carbohydrates, as well as vitamins A, C, E and F [11]. Activated carbons were prepared from walnut shells. Foliage, barks, roots and green shells are used for dyeing fabrics (wool, silk, yarn), as well as for hair coloring [9, p.212-214]. Vitamin concentrate is prepared from immature fruits [3; 12 p.54]. Therefore, it is necessary to conduct a study of the chemical compositions of leaves and immature fruits to search for an additional source of medicinal plants. Additionally, species of the genus *Juglans L*

are moisture-loving plants, but they differ in drought resistance [13]. Drought resistance is determined in the driest growing season [14]. We use physiological methods to identify drought resistance features of the species of the genus *Juglans L* in the conditions of the Bryansk region.

Materials and methods

The leaves and immature fruits of four species of the genus *Juglans L* were used as research materials: walnut (*Juglans regia*), Manchurian walnut (*Juglans mandshurica*), black walnut (*Juglans nigra*), butternut (*Juglans cinerea*). Anatomical study and elemental compositions of the leaves and immature fruits were carried out using the ion-scanning electron microscope. Anatomy and morphological parameters of the epidermal cells of

the leaves of various species of the genus *Juglans* L were studied using the SEM Quanta 200 3D, the Carl Zeiss microscope and the program VideoTest 5.0. Chemical compositions of the leaves and immature fruits were analyzed following the instructions in GOST 5900-73 (for dry matter and water content), differential spectrophotometry (for flavonoid content), in titrimetry (content of ascorbic acid; content of free organic acids), in the Levantalya method (for determination of antioxidant activity of plant materials), in spectrophotometry (for chlorophyll content) in refractometer (for content of free and bound water). Drought resistance was evaluated by the degree of damage of the leaves.

Results and discussions

1. The chemical composition of leaves and immature fruits of some species of the genus *Juglans* L in the conditions of Bryansk region.

The data obtained allows us to conclude that the leaves of four species of the genus *Juglans* L contain a large amount of magnesium, which has a calming effect on the brain, and a large amount of this macro-element promotes anti-stress action [9, p.33-44]. The content of magnesium 1.18% (Table -1) is determined in the walnut *Juglans nigra*. The leaves of the four species of the genus *Juglans* L also contain a large amount of calcium. It is well known that the species of the genus *Juglans* L have the properties of drought resistance and winter hardiness [10], which are enhanced due to the presence of high calcium content.

Table 1

Elemental composition of the leaves of four species of the genus *Juglans* L (Wt%)

Element	<i>Juglans regia</i>	<i>J.nigra</i>	<i>J.mandshurica</i>	<i>J.cinerea</i>
C	46.34	42.65	40.75	61.93
O	48.60	53.92	55.89	36.01
Mg	0.92	1.18	0.84	0.28
Al	-	0.17	0.15	0.13
P	0.15	0.18	-	-
K	0.83	0.75	1.02	0.51
Ca	0.80	1.15	0.96	0.94
Si	2.35	-	0.39	0.19

This is explained by the role of calcium because calcium and magnesium are the basis of pectin of medial plates of cells (intercellular substance) in the plant body. Calcium ensures the integrity of cell membranes has a water retention capacity of protoplasm, involves in the construction of the cell wall of plants; increases plant tissues' hardness and improves plants' endurance. Its contents are 0.15% (Table-1), which is found in the black walnut leaves and 4.03% in the Manchurian walnut immature fruits (Table 2). It is known that calcium is an essential element in the human body. It plays an important role in the regulation of the blood clotting, in the heart rhythm regulation. It also plays a vital role in immune processes, in the synthesis and enzyme works [11]. Being present in every cell of the body, calcium regulates the reproduction of cells and protein synthesis. For these reasons, it is important to find affordable medicinal raw materials with a high content of calcium. A large amount of potassium is also found in the leaves and immature fruits. The content of potassium is 0.51- 1.02% (Table 1) in the

leaves and its content is revealed from 2.55% - 3.87% (Table-2) in the immature fruit. Potassium is involved in the regulation of cellular osmotic potential. Potassium plays an important role in the drought resistance and winter hardiness. It also plays an essential role in the regulation of water balance and the process of normalization of cardiac rhythm [9, p.33-44]. It can be suggested that the leaves and immature fruits of the genus *Juglans* L are an available source of calcium, potassium and magnesium due to the high content of potassium according to our data (Table 1-2). Silicon is found in all four species of the genus *Juglans* L and its content is up to 2.34% in the leaves of *Juglans regia* that increases plant resistance to powdery mildew. Iodine is also found in the immature fruits. Tincture of pericarp is recommended in the treatment of hyperthyroidism [7, p.121-123].

Table 2

Elemental compositions of immature fruits of four species of the genus *Juglans L* (Wt%)

Element	<i>Juglans regia</i>	<i>J.nigra</i>	<i>J.mandshurica</i>	<i>J.cinerea</i>
C	56.71	52.84	52.08	56.70
O	38.08	42.22	38.82	38.54
Mg	0.28	0.12	0.55	0.21
Al	0.03	0.17	0.16	0.09
P	0.52	0.28	0.20	0.40
K	3.87	3.34	2.85	2.55
Ca	-	0.44	4.03	1.23
Si	0.08	0.13	0.08	0.05
S	0.27	0.21	0.19	0.23
I	0.14	0.24	0.86	-

The data obtained allow us to conclude that the leaves and immature fruits of all species of the genus *Juglans* growing in the Bryansk region contain

various biological compounds: flavonoids, organic acids (up to 3,838% in immature fruits of the walnut *Juglans mandshurica*), ascorbic acid (Table 3-4).

Table 3

The quantitative content of several compounds in the leaves of four species of the genus *Juglans L*

Class of substances	<i>Juglans regia</i>	<i>Juglans nigra</i>	<i>Juglans mandshurica</i>	<i>Juglans cinerea</i>
Free water (%)	56.345±3.229	41.575±4.962	51.639±1.648	45.610±1.514
Bound water (%)	15.975±2.353	18.705±1.414	19.112±1.868	17.818±1.707
Dry matter (%)	27.679±1.228	34.720±4.962	29.248±1.689	36.571±1.5149
Flavonoid (%)	2.25 ±0.08	1.424±0.09	1.894±0.10	2.351±0.07
Organic acid (%)	3.474	3.334	3.824	3.012
Ascorbic acid (%)	0.156±0.012	0.104±0.007	0.119±0.019	0.061±0.008
Antioxidant activity (мг/л)	224	288	184	114

Table 4

The quantitative content of several compounds in the immature fruits of four species of the genus *Juglans L*

Class of substances	<i>Juglans regia</i>	<i>Juglans nigra</i>	<i>Juglans mandshurica</i>	<i>Juglans cinerea</i>
Free water (%)	59.629±2.911	42.710±2.763	41.427±5.298	43.729±1.474
Bound water (%)	27.563±1.256	35.250±1.386	45.114±2.023	38.589±1.253
Dry matter (%)	12.807±1.911	22.039±2.763	13.458±1.298	17.681±1.474
Flavonoid (%)	1.689±0.12	1.857±0.08	2.051±0.11	1.652±0.08
Organic acid (%)	3.828	2.148	3.838	2.675
Ascorbic acid (%)	0.765±0.027	0.811±0.018	0.117±0.018	0.745±0.017

Immature fruits of the walnut *Juglans mandshurica* contain a small amount of vitamin C. Fruits of other species contain sufficiently high amount of vitamin C and 0,811% vitamin C is found

in immature fruits of the walnut *Juglans nigra*. To conclude that, unripe fruits of various species of the genus *Juglans L* can be used as a source of vitamin C to make jams and fortified wine [14]. The high

content of bound water in the leaves and unripe fruits increases their resistance to unfavorable factors. Animal eating flavonoid-containing leaves have hypotensive, antispasmodic and anti-inflammatory effects [15].

2. Drought resistance of some species of the genus *Juglans* L. in the conditions of Bryansk oblast.

Stomata play an important role in adaptation processes in different conditions and also in the plant productivity. The stomatal apparatus of four species of the genus *Juglans* L has an anomotsitny type. Stomata are randomly arranged on the leaf surface. Guard cells dominated the principal cells of the epidermis is observed on the leaf surface of the walnut *Juglans mandchurica*. Stomata of the walnut *Juglans regia*, *Juglans nigra*, *Juglans cinerea* are located in the same level with the main cells of the epidermis (Fig. 1 to 4). Additionally, stomata are found only on the lower side of the leaf. The number

of stomata per unit area on the leaf surface varies between 81- 115. It depends on the type species. The highest number of stomata is observed on the leaf surface of the walnut *Juglans cinerea*, they also have the largest size of stomata (Table 5).

According to data obtained by studying of the structural features of the epidermis of the leaf four species of the genus *Juglans* L that cells of the abaxial side are smaller than the cells of the adaxial side of the leaf (Table 6). The shape of the upper epidermal cells is: quadrilateral and polygonal. These cells are densely arranged to each other, and the cells of the lower epidermis have a substantially quadrangular shape. The cells of the upper epidermis *Juglans regia* mostly have a quadrangular shape. The length of the epidermal cells is greater than their width. Two types of trichomes were found: simple and glandular trichomes. Glandular trichomes are found only on the veins.

Table 5
Morphological parameters of the stomata of the lower epidermis of the leaf of four species of the genus *Juglans* L: * Accuracy in comparison with the *Juglans regia* (by Student's test (p < 0.05).

Specie	Area(μm^2)	Perimeter (μm)	Length (μm)	Width (μm)	Number of stomata /1MM ²
<i>Juglans regia</i>	300.10±7.26	60.84±2.28	23.10±2.90	16.54±3.86	89
<i>Juglans nigra</i>	239.72±4.85*	50.51±3.28	20.34±3.84	14.87±2.53	98
<i>Juglans mandchurica</i>	228.48±15.71*	49.68±6.87*	20.13±2.44	14,70±1.62	115*
<i>Juglans cinerea</i>	315.83±14.99	66.56±8.39	22.59±3.89	17.89±2.56	81

Table 6
Morphological parameters of the epidermal cells of the leaf of four species of the genus *Juglans* L: * Accuracy in comparison with the *Juglans regia* (by Student's test (p < 0.05).

Specie	Cell	Area(μm^2)	Length (μm)	Width (μm)
<i>Juglans regia</i>	Upper epidermis	289.25±28.39	25.53±3.86	18.09±2.08
	Lower epidermis	189.73±20.96	20.39±1.77	14.53±3.93
<i>Juglans nigra</i>	Upper epidermis	326.59±22.82*	28.72±5.54	18.75±3.49
	Lower epidermis	194.27±48.43*	21.52±4.63	13.36±1.68
<i>Juglans mandchurica</i>	Upper epidermis	319.19±20.52	28.89±3.58	19.05±3.75
	Lower epidermis	191.32±27.26	17.94±5.72	14.64±4.77
<i>Juglans cinerea</i>	Upper epidermis	300.89±20.82	25.89±4.75	20.75±2.89
	Lower epidermis	184.57±40.23	18.72±6.83	12.39±2.78

The study of leaf morphology of some species of the genus *Juglans* L in the conditions of Bryansk region showed that leaf of the walnut *Juglans nigra*

has the smallest size, while the leaf of the walnut *Juglans mandchurica* has the largest size (Table-7).

Table 7
Morphological parameters of the leaf of four species of the genus *Juglans* L: * Accuracy in comparison with the *Juglans regia* (by Student's test ($p < 0.05$)).

Specie	<i>Juglans regia</i>	<i>Juglans nigra</i>	<i>Juglans mandchurica</i>	<i>Juglans cinerea</i>
Leaf length(cm)	40.5±4.14	40.75±4.13	61.4 ± 3.20*	43.7 ± 2.99
Leaf width (cm)	28.8±2.70	23.67± 1.53	32.4 ± 2.30*	21.24 ± 1.87
Leaflet length (cm)	14.17±3.68	9.92±1.81	15.22± 2.87	10.45± 1.78
Leaflet width(cm)	6.01±1.66	2.91±1.24*	5.76± 1.23	4.73± 0.62
Leaflet area (cm ²)	58.17±4.20	17.67±3.25*	61.44±6.28	34.95±5.68*
Number of leaflets	7 - 9	19-21	15-17	15-17
Petiole length (cm)	9.3± 2.16	5.58±0.86	10.8± 2.04	5.6 ± 0.8

Qualitative and quantitative composition of pigments is considered as indicator of plant adaptation to environmental conditions. The amount of chlorophyll and carotenoid of four species of the genus *Juglans* L is decreased; this is due to adaptation of plants to high temperature and water

shortages (Table-8). However, the amount of bound water is increased. The amount of bound water of the walnut *Juglans mandchurica* is not practically changed; it can be explained by the fact that their leaves are damaged by drought.

Table 8
Content of chlorophylls and carotenoids in the leaves of four species of the genus *Juglans* L.

	Specie	<i>Juglans regia</i>	<i>Juglans nigra</i>	<i>Juglans mandchurica</i>	<i>Juglans cinerea</i>
Chlorophyll a (mg / g)	25.06	1.05	1.25	0.85	0.89
	18.07	0.61	0.72	0.56	0.59
Chlorophyll b (mg / g)	25.06	0.37	0.35	0.34	0.33
	18.07	0.25	0.27	0.31	0.21
Chlorophyll. a+ b (mg / g)	25.06	1.42	1.60	1.19	1.22
	18.07	0.86	0.99	0.87	0.80
Carotenoid (mg / g)	25.06	0.28	0.22	0.19	0.18
	18.07	0.12	0.14	0.11	0.11
Free water (%)	25.06	56.35±3.23	41.57±4.96	51.64±1.65	45.61±1.51
	18.07	45.73±2.14	35.26±2.36	40.30±1.88	38.11±1.58
Bound water (%)	25.06	15.97±2.35	18.71±1.41	19.11±0.87	17.82±1.71
	18.07	25.86±3.02	29.89±1.77	20.52±1.78	22.58±2.36

The results obtained from (Table-9) shows that the walnut *Juglans mandchurica* and *Juglans cinerea* are the greatest water loss but they are the least drought - resistant plants. Drought resistance was evaluated by the extent of the damage of the leaf on

the four-point scale: 0 - No damage; 1 - Damage of the edges of leaves; 2- Damage to a significant part of the leaves (about half); 3- Damage to all leaves [1; 2].

Table 9
Characteristics of water-holding capacity of leaves and the relative extent of drought resistance of four species of the genus *Juglan* L.

	Water lost after leaf wilting , % of water content			Drought resistance, point
	After 2 h.	After 4h.	After 6h.	
<i>Juglans regia</i>	8.05	17.23	24.35	0
<i>Juglans nigra</i>	7.51	18.56	23.24	0
<i>Juglans mandchurica</i>	20.25	45.67	65.35	1
<i>Juglans cinerea</i>	15.25	28.77	55.11	1

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Conclusions

1. Leaves and immature fruits of four species of the genus *Juglans* L are highly valued due to their high content of calcium and potassium, magnesium, and vitamin C and high antioxidant activity. In conclusion, that they can be used as sources of additional medicinal herbs.

2. Four species of the genus *Juglans* L are drought resistant and winter resistant plants in the conditions of Bryansk region, due to high contain of calcium, potassium and magnesium.

3 The walnut *Juglans regia*, *Juglans nigra* are the most drought-resistant plants in the conditions of Bryansk region.

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