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## The material relating to the anatomy of *Rhaponticum serratuloides* leaf blades according to age conditions

In the article the features of the morphological and anatomical structure of leaf blades in the process of ontogenesis of *Rhaponticum serratuloides* Georgi (Bohr.) during juvenile, immature, virginal, generative and senile age states were presented. It was noted that as the plant matures, the degree of dissection and linear dimensions of the leaf blades, as well as their vascular bundles and trichomes, increase. It was established that in the juvenile and immature periods of development whole and elliptical leaves predominate. In the virginal state, in addition to the typical entire leaves, leaves with an unpaired pinnate leaf blade appear. The generative state of *Rh. serratuloides* is characterized by having upper leaves as entire, sessile; the lower ones are petiolate, pinnately lobed with a large apical lobe. In the senile state, leaves of immature and virginal types are found. General patterns of leaf internal structure of *Rh. serratuloides* and another representative of the same genus *Rh. carthamoides* Willd (Iljin) were revealed as following: the plants are of dorsolventral type of leaf blade structure, with collateral vascular bundles of a closed type with a sclerenchyma covering and leaf pubescence. Along with this, characteristic diagnostic features for leaves of *Rh. serratuloides* were described as the following: the absence of capitate glands and long cord-like hairs during all age states, the presence of air-bearing cavities in the parenchyma of virginal and senile plants, associated with the habitat of the plant under study, because it grows in flooded meadows, along the shores of lakes and swamps.

**Keywords:** age states, anatomical features, types of leaf blades, *Rh. serratuloides*, *Rh. carthamoides*.

### Introduction

Among the species of the genus *Rhaponticum*, *Rh. carthamoides* is perhaps the most economically important one, as its roots, rhizomes and leaves are included in the State Pharmacopoeia of the Russian Federation [1] and the Republic of Belarus [2]. This prompted a detailed study of the anatomical characteristics of various parts of this plant, including the anatomical features and ultrastructure of all vegetative organs and secretory elements of *Rh. carthamoides* [3]. Anatomical and morphological structure of leaves and stems of another species *Rh. karatavicum* was investigated by A.K. Berkenov [4].

Anatomical study of *Rh. serratuloides* was initiated by G.Zh. Sultangazina [5], who first identified the diagnostic characteristics of medicinal raw materials of this species in comparison with *Rh. carthamoides*. Also for the same purposes, A.V. Kotylevskaya and others [6] studied the petiolar characteristics of the petiole of *Rh. serratuloides*.

It should be noted that in Sultangazina's work, the anatomical characteristics of the vegetative (leaves and stems) and generative (flower and involucre leaves) organs of *Rh. serratuloides* were studied during 1, 4 and 5 years of the vegetation year, i.e. virginal and generative individuals.

Our goal is to research *Rh. serratuloides* leaf blades throughout the entire ontogeny, with the exception of the seedling stage. Study of the morphological and anatomical structure of *Rh. serratuloides* leaves will allow us to identify general and individual characteristics of the species under study in comparison with other representatives of this genus, determine some patterns of its ontogenesis, and also supplement information about the species diagnostic characters of *Rh. serratuloides*.

### Materials and Methods

The object of the study is the leaves of *Rhaponticum serratuloides* of various age states: j — juvenile; im — immature; v — virginal; g — generative; s — senile (except for seedlings), collected in the Ak-mola and Karaganda regions during the fruiting phase. Sampling (Table 1) was carried out from populations growing in flooded, grass-

wormwood, swampy meadows, roadside hollows in the vicinity of Karaganda city, Karabas, Abay villages in the Karaganda region, in the vicinity of Astana city and the Korgalzhinsky reserve in the Akmola region.

Table 1

## Research points

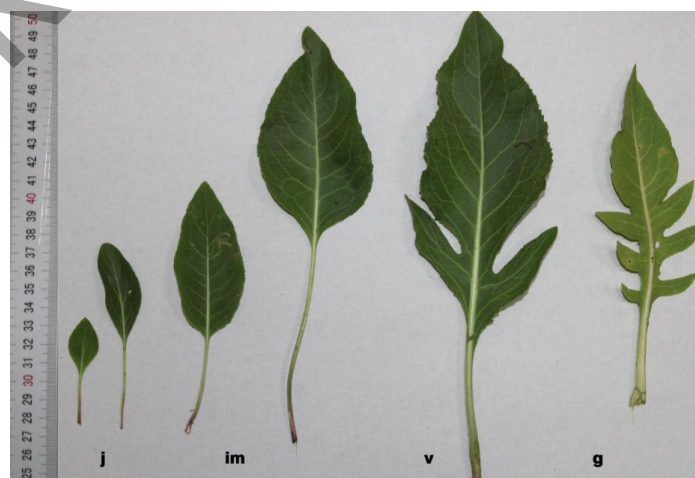
Number	Growing point	Coordinates		
		N	E	Height above sea level
1.	Karaganda region, Abay reg.	49°34'48.0»	72°53'38.1»	503
2.	Karaganda region, Abay reg.	49°38'03.4»	72°50'38.3»	500
3.	Karaganda region, Abay reg. (between Abay and Saran)	49°40'57.6»	72°51'37.2»	485
4.	Akmola region, Astana. Neighborhood of the village Karazhar, Nura River valley, Astana-Malinovka highway	51°05'27.9»	71°11'05.3''	341
5.	Akmola region, Korgalzhinsky reserve, shore of the lake Yesey	50°32'08.5»	69°39'03.4''	305

Features of age-related conditions of *Rh. serratuloides* were studied in situ according to the methodological instructions of T.A. Rabotnov [7], A.A. Uranov [8], O.V. Smirnova, et al. [9]. To determine the average values of morphometric parameters of the leaf blade, 10 samples of each shoot were taken. The length and width of the leaf blade were measured using an electronic caliper with digital indexing. To study the anatomic structure, the selected leaves were fixed in a mixture of glycerol: 96 % alcohol: distilled water in a ratio of 1:1:1 (Straus-Fleming mixture) [10, 11]. Transverse sections of the sheet were made by hand. For the leaf, micropreparations were made from the central part of a fragment of the middle fox. Sections were cleared with glycerol. The preparations were photographed on an Altami microscope with a 3.1 megapixel digital camera, with magnification of 16x4 and 16x10. A blue filter was used in some photographs. Photo processing and microscopic measurements were performed in the Altami Studio program using Paint 10.0. When describing the anatomical structure, we used the principles set out in the works of N.A. Aneli [12], L.I. Lotova [13], P.J. Rudall [14]. For each sample, at least 10 micropreparations (microscope slides) were made.

Statistical processing of the results of morphometry and anatomy of leaf blades was carried out using the Statistica 7.0 program.

## Results and Discussion

*Morphological features of leaves.* During ontogenesis, the leaf blades of *Rh. serratuloides* undergo a number of changes (Fig. 1.). The first true leaves are narrow-lanceolate. During the juvenile and immature periods of development, the leaves are entire, elliptical, and glabrous with a long petiole. Since the virginal state, in addition to the typical entire leaves, a dissection of the leaf blade appears with the formation of one large and 2-3 small lobes (Table 2). During generative state *Rh. serratuloides* is characterized by various forms of leaves: the upper ones are entire, sessile, the lower ones are petiolate, and its blade is toothed, pinnately divided at the base, with 1–3 pairs of oblong or almost lanceolate lobes. During senile state, the leaves are the same of immature and virginal types.

Figure 1. Appearance of *Rhaponticum serratuloides* leaves of different age states

**Morphometric parameters of *Rhaponiticum serratuloides* leaves during ontogenesis (in cm)**

Age conditions	Leaf blade length		Leaf blade width	
	Simple	Dissected	Simple	Dissected
Juvenile	3.86±0.52 Cv 23.2	-	1.47±0.52 Cv 61.1	-
Immature	7.66±0.44 Cv 16.2	-	1.76±0.19 Cv 30.8	-
Virginal	15.91±0.98 Cv 17.5	-	5.5±0.34 Cv 17.7	-
Generative	11.99±0.41 Cv 21.73	15.65 ±0.41 Cv 17.8	5.91±0.27 Cv 29.68	6.79±0.25 Cv 25.14

*Anatomical structure of a leaf.* In a cross section, the leaf is flat, and of dorso-ventral type (Fig. 2, 3). The leaf veins protrude significantly from the underside of the leaf. The leaf is surrounded on both sides by cells of the upper and lower epidermis; the shape is clearly shaped, with thickened outer walls. On the surface of the epidermis on the lower side, mainly in the area of the midrib, only simple multicellular trichomes are located. In a study by G. Sultangazina [5], it is noted that in virginal leaves, on both surfaces of the epidermis, and in generative plants, mainly on the upper epidermis, small capitate hairs with a secretory unicellular head are also found. Columnar mesophyll consists of 2 cell layers, spongy mesophyll is multilayered. The conducting bundle is collateral, of closed type. The central beam is ovoid, the side beams are oval. The xylem strand is oriented towards the upper epidermis, the phloem is towards the lower side. The vascular bundle is intertwined with sclerenchyma on both sides. A section of lamellar collenchyma is localized above the central vascular bundle under the epidermis. During the senile age state, the separation of the lower and upper epidermis from the leaf parenchyma with the formation of air cavities is observed. In Sultangazina's studies [5], this process was observed in leaves of the 1st year of vegetation, i.e. virginal.

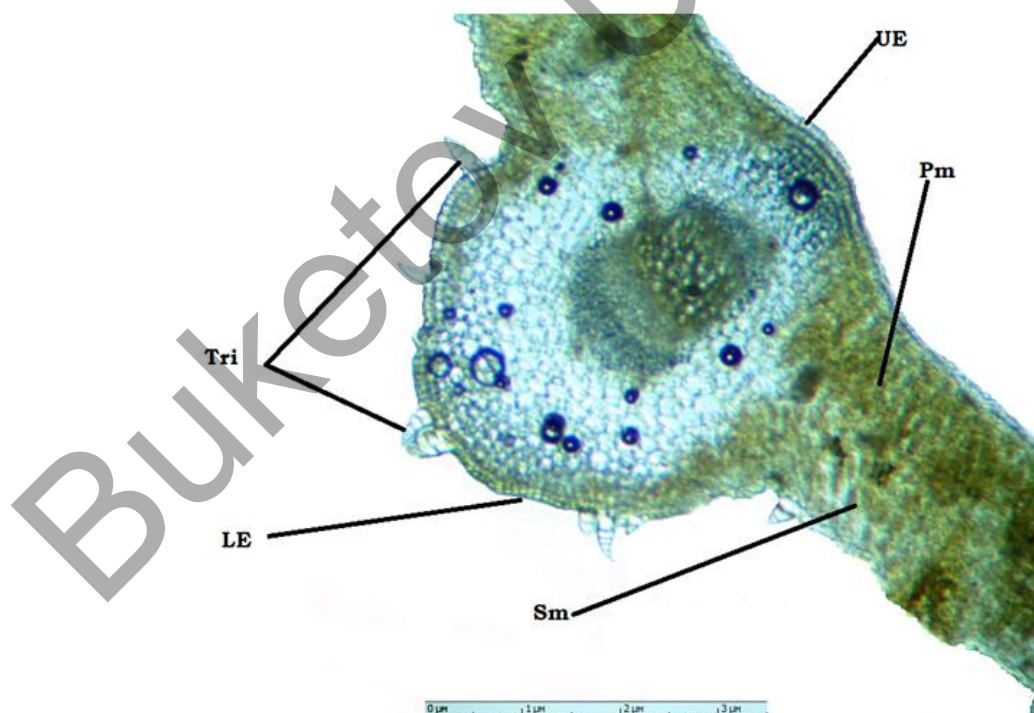


Figure 2. Anatomical structure of a cross section of a *Rh. serratuloides* leaf (the fragment is from the midrib area of the terminal leaflet of the generative state plant, magnified to 16x20): UE — upper epidermis, LE — lower epidermis, Tri — trichomes, PM — columnar mesophyll, SM — spongy mesophyll

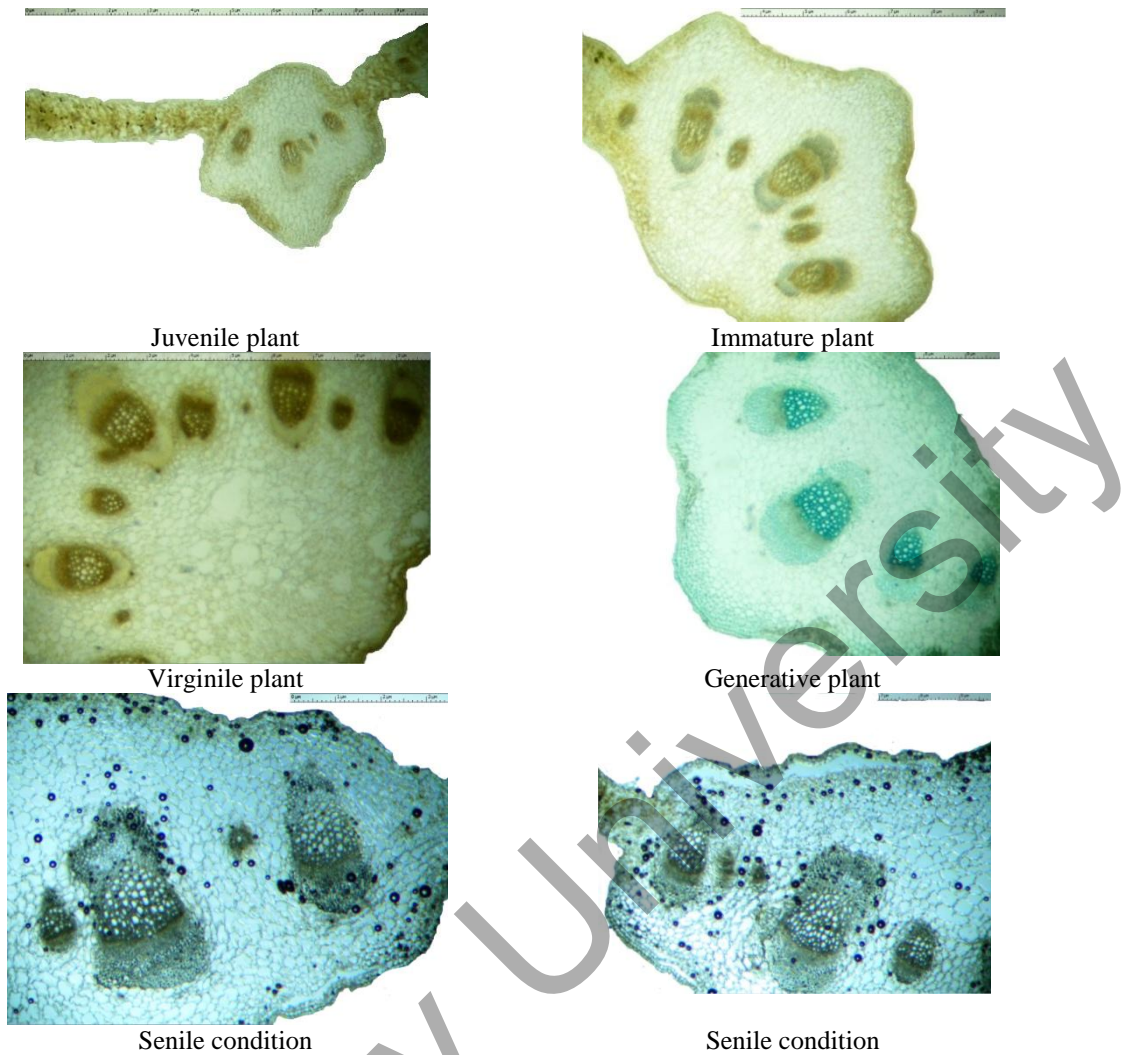


Figure 3. Cross sections of *Rh. serratuloides* leaves in different age states (magnitude 16x20)

Features of the anatomical indicators of leaf blades as *Rh. serratuloides* is growing and developing are shown in Table 3.

Table 3

**Quantitative parameters of individual cells and tissues of the *Rh. serratuloides* leaf during ontogeny (in microns)**

Indicators	Age states of <i>Rh. serratuloides</i>				
	Juvenile	Immature	Virginal	Generative	Senile
Leaf thickness at midrib	4.80±0.02	7.64±0.02	12.10±0.31	7.35±0.18	11.18±0.07
Leaf side width	1.25±0.08	2.22±0.02	1.73±0.08	2.33±0.22	1.49±0.09
Conductive beam length	1.33±0.02	2.09±0.04	2.74±0.05	3.16±0.08	4.4±0.08
Conductive beam width	0.6±0.02	0.9±0.09	1.41±0.05	1.65±0.09	2.11±0.03
Thickness of the lower epidermis	0.06±0.003	0.084±0.002	0.13±0.001	0.098±0.01	0.09±0.01
Upper epidermis thickness	0.07±0.002	0.086±0.007	0.12±0.002	0.12±0.004	0.12±0.008
Trichome length	0.31±0.02	0.31±0.02	0.74±0.06	0.68±0.07	0.8±0.01

Analysis of the anatomical structure of *Rh. serratuloides* leaf has shown that as the plant is growing and developing, the leaf blades are characterized by an increase in the linear dimensions of vascular bundles and trichomes. In the virginal and senile stages of development, the leaves reach their maximum thickness in the midrib area. The maximum leaf width in the lateral part is observed in *Rh. serratuloides* in immature and

generative age states. No significant differences were found between the cells of the lower and upper epidermis of juvenile and immature individuals. In generative and senile leaves, the cells of the lower epidermis are somewhat smaller than the upper ones.

### Conclusion

The study made it possible to identify the features of the anatomical and morphological structure of leaves in the process of ontogenesis. As the plant matures, the degree of dissection and linear dimensions of the leaf blades, as well as their vascular bundles and trichomes, are increasing.

In the juvenile and immature state, the leaves are entire, narrow-lanceolate and elliptical with a long petiole. Since the virginal state, leaflets appear as unpaired and pinnately divided, which consist of one large and 2-3 small lobes. In the generative state, *Rh. serratuloides* is characterized by various forms of leaf blades: the upper ones are simple, sessile, the lower leaves are entire, pinnately divided at the base, with 1–3 pairs of oblong or almost lanceolate lobes. In the senile state, leaves of immature and virginal types are observed.

The anatomical structure of *Rh. serratuloides* leaf blades was compared to *Rh. carthamoides*. The comparative analysis has revealed some characteristics: it is the absence of pubescence on *Rh. carthamoides*. On the lower epidermis only simple multi-cellular hairs (trichomes) are observed in the midrib area. The location of the conductive bundles depends on the age-related condition. In the leaves of virginal and senile individuals, large vascular bundles alternate with small ones. In the juvenile, immature and generative stages there are 3 central large bundles; small bundles may be located between them. Also, for virginal and senile plants, the presence of air-bearing cavities in the leaf parenchyma was noted; this is associated with the habitat, because *Rh. serratuloides* grows in flooded meadows, along the shores of lakes and swamps.

### Acknowledgements

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### ***Rhaponticum serratuloides* жастық күйлері бойынша жапырақ тақталарының анатомиясына арналған мәліметтер**

Мақалада *Rhaponticum serratuloides* Georgi (Bobr.) онтогенез процесіндегі: ювенильді, иматурлы, виргинильді, генеративтік және сенильді жастық күйлері бойынша жапырақ тақталарының морфологиялық және анатомиялық құрылымының ерекшеліктері берілген. Өсімдік жетілген сайын жапырақ тақталарының, сондай-ақ олардың өткізгіш шоқтарымен трихомаларының бөліну дәрежесімен сызықтық өлшемдері артады. Ювенильді және иматурлы даму кезеңдерінде тұтас, эллипс тәрізді жапырақтардың басым болатыны анықталды. Виргинильді күйде әдеттегі тұтас жапырақтардан басқа, жұпталмаған, яғни қауырсын тәрізді бөлінген жапырақ тақтасы бар жапырақтар пайда болады. *Rh. serratuloides* генеративті күйіндегі жапырақты сипаттасак: жоғарғысы — тұтас, сағақсыз; төменгілері сағақты, жоғарғы жағы үлкен қауырсын тәрізді бөлінген. Сенильді жағдайында жетілмеген және қызғылт түрдегі жапырақтар кездеседі. *Rh. serratuloides* жапырақтарының ішкі құрылысының жалпы заңдылықтары осы туыстың басқа өкілімен, яғни *Rh. carthamoides* Willd. (Pjin) бірге анықталды: жапырақ тақтасының дорсовентральды құрылымы, склеренхималық жабындысы бар тұйық типті коллатеральды тамыр шоғырлары, жапырақтың түтіктері. Сонымен қатар, тек *Rh. serratuloides* жапырақтарына тән диагностикалық белгілер байқалады: қарастырылған барлық жас жағдайларында бас бездері мен ұзын сым тәрізді түтктердің болмауы, сондай-ақ зерттелетін өсімдіктің тіршілік ету ортасымен байланысты виргинильді және сенильді өсімдіктердің паренхимасында ауа қуыстарының болуы, өйткені ол су басқан шалғындарда, көлдер мен батпақтардың жағасында өседі.

*Кілт сөздер:* жастық күйлер, анатомиялық ерекшеліктері, жапырақ тақталарының түрлері, *Rh. serratuloides*, *Rh. carthamoides*.

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### **Материал к анатомии листовых пластинок *Rhaponticum serratuloides* по возрастным состояниям**

В статье представлены особенности морфологического и анатомического строения листовых пластинок в процессе онтогенеза *Rhaponticum serratuloides* Georgi (Bobr.): ювенильного, имматурного, виргинильного, генеративного и сенильного возрастных состояниях. Отмечено, что по мере взросления растения увеличиваются степень рассеченности и линейные размеры листовых пластинок, а также их проводящих пучков и трихом. Установлено, что в ювенильном и имматурном периодах развития преобладают цельные, эллиптические листья. В виргинильном состоянии, кроме типичных цельнокрайних листьев, появляются листья с непарно перистораздельной листовой пластинкой. Для генеративного состояния *Rh. serratuloides* характерны верхние — цельные, сидячие; нижние — черешковые, перистолопастные с крупной верхушечной долей. В сенильном состоянии встречаются листья имматурного и виргинильного типов. Выявлены также общие закономерности внутреннего строения листьев *Rh. serratuloides* с другим представителем этого рода, *Rh. carthamoides* Willd. (Pjin): дорзовентральный тип строения листовой пластинки, коллатеральные проводящие пучки закрытого типа со склеренхимной обкладкой и опушение листьев. Наряду с этим отмечены диагностические признаки, характерные только для листьев *Rh. serratuloides*: отсутствие головчатых железок и длинных шнуровидных волосков во всех рассмотренных возрастных состояниях, а также наличие воздухоносных полостей в паренхиме виргинильных и сенильных растений, связанное со средой обитания изучаемого растения, так как оно произрастает на заливных лугах, по берегам озер и болот.

*Ключевые слова:* возрастные состояния, анатомические особенности, типы листовых пластинок, *Rh. serratuloides*, *Rh. carthamoides*.

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