IRSTI 34.29.35 https://doi.org/10.26577/eb.2020.v84.i3.02

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BIOCHEMICAL AND ANATOMICAL RESEARCH OF KAZAKHSTAN *SERIPHIDIUM HEPTAPOTAMICUM* (ASTERACEAE)

Currently, the study of the structure and composition of plants is very important for medicine, perfumery, farms, etc. The section of the Asteraceae family includes Seriphidium heptapotamicum (Poljak) Ling & Y.R. Ling. This study discusses biochemical and anatomical features. According to new observations, the the previous description of the species was added by the additional information. Anatomical studies were carried out by the vegetative organs of S.heptapotamicum using the paraffin method. The anatomy of S.heptapotamicum have not been studied. Anatomically, the root cross section has the epidermis as a protective tissue in the outer layer and the endodermis is not clearly seen. The stems have a thick, well-developed layer of sclerenchyma. Leaves are equilateral. For a biochemical study, the essential oil was isolated from the aerial parts of S.heptapotamicum using a Clevenger type apparatus. The yield of S.heptapotamicum essential oil was found to be 2.4 %. The chemical composition of the essential oil was analyzed by GC-FID and GC-MS simultaneously. The chemical composition of essential oil depends on the collectionsites. The essential oil is composed of 14 components, in which the major dominant constituents were Thujone-41.10%, 1,8-Cineole-22.84%, β-Thujone-17.45% and Camphor (11.99%). In addition, the essential oil contents of S. heptapotamica have been obtained in this study.

Key words: Seriphidium heptapotamicum, Altyn-Emel, Essential Oil, GC; GC-MS.

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Қазақстандағы *Seriphidium heptapotamicum* (Asteraceae) өсімдігінің биохимиясы мен анатомиялық құрылысын зерттеу

Қазіргі кезде өсімдіктің құрылысы мен құрамын зерттеу медицина, парфюмерия, фермер шаруашылығы және т.б. салалар үшін өте маңызды. Seriphidium heptapotamicum (Poljak) Ling & Y.R. Ling. өсімдігі күрделігүлділер тұқымдасына жатады. Бұл мақалада Seriphidium heptapotamicum өсімдігінің биохимиялық және анатомиялық ерекшеліктері талқыланды. Жаңа зерттеулерге сәйкес түрдің алдыңғы сипаттамаларына қосымша ақпарат берілді. Seriphidium heptapotamicum өсімдігінің вегетативті мүшелеріне (тамыр, сабақ, жапырақ) анатомиялық зерттеу «Парафин» әдісін қолдану арқылы жүргізілді. Seriphidium heptapotamicum өсімдігінің анатомиялық құрылысы осы уақытқа дейін зерттелмегендігі анықталды. Өсімдік тамырының көлденең кесіндісінің анатомиялық құрылысында эпидермис қорғаушы ұлпа ретінде сыртқы қабатын қаптайды, ал эндодерма анық көрінбейді. Сабақта склеренхима қабатының жақсы дамығаны анықталды. Жапырағы тең қабырғалы. Seriphidium heptapotamicum өсімдігіне биохимиялық зерттеу жүргізілді және өсімдіктің жерүсті бөліктерінен Кливенджер аппаратының көмегімен эфир майы алынды. Нәтижесінде шыққан эфир майы 2,4%-ды құрады. Эфир майының химиялық құрамына GC-FID және GC-MS аппаратының көмегі арқылы талдау жасалды. Бұл зерттеуде Seriphidium heptapotamicum өсімдігіндегі эфир майының құрамы алынып, эфир майы 14 компоненттен тұратындығы және ондағы негізгі доминантты компоненттер туйон-41,10%, 1,8-цинеол-22,84%, β-туйон-17,45%, камфор-11,99% екендігі анықталды.

Түйін сөздер: Seriphidium heptapotamicum, Altyn-Emel, эфир майы, GC; GC-MS.

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Биохимические и анатомические исследования
***Seriphidium heptapotamicum* (Asteraceae) в Казахстане**

В настоящее время изучение структуры и состава растений очень важно для медицины, парфюмерии, фермерских хозяйств и т.д. Seriphidium heptapotamicum (Poljak) Ling & Y.R. Ling. входит в раздел семейства сложноцветных. В этом исследовании обсуждаются биохимические и анатомические особенности. Согласно новым наблюдениям, предыдущее описание вида было дополнено дополнительной информацией. Анатомические исследования проводились на вегетативных органах Seriphidium heptapotamicum с использованием парафинового метода. Анатомия Seriphidium heptapotamicum не изучена. Анатомически, поперечное сечение корня имеет эпидермис в качестве защитной ткани во внешнем слое и эндодерма четко не видна. Стебли имеют толстый, хорошо развитый слой склеренхимы. Листья равносторонние. Для биохимического исследования эфирное масло было выделено из надземных частей Seriphidium heptapotamicum с использованием аппарата Кливенджера. Выход эфирного масла Seriphidium heptapotamicum составил 2,4%. Химический состав эфирного масла анализировали с помощью GC-FID и GC-MS одновременно. Химический состав эфирного масла зависит от коллекций. Эфирное масло состоит из 14 компонентов, в которых основными доминирующими компонентами были туйон-41,10%, 1,8-цинеол-22,84%, β-туйон-17,45% и камфора – 11,99%. Кроме того, в этом исследовании было получено содержание эфирного масла Seriphidium heptapotamicum.

Ключевые слова: Seriphidium heptapotamicum, Алтын-Эмель, эфирное масло, GC; GC-MS.

Introduction

Kazakhstan flora is rich in economically important kinds of plants. In Kazakhstan there are about 6000 species, 1120 genera and 160 families (Ivaschenko, 2006). More than 700 species are endemic and no less than 1406 species of medicinal plants are the members of 612 genera belong to 134 families (Grudzinskaya et al., 2014).

Asteraceae includes over 32000 currently accepted species, in over 1900 genera in 13 subfamilies. The family Asteraceae has about 1186 species, of which almost 196 are endemic, and about 130 are medicinal in Kazakhstan (The Plant List, 2016; Ivaschenko, 2006).

The genus *Artemisia* (Astraceae) consists of about 500 species, occurring throughout the world (Bora & Sharma, 2011). The species of the genus are spread throughout the Northern Hemisphere's temperate zones with few members in the Southern Hemisphere (Valles et al., 2005). The most commonly accepted subdivisions of Artemisia are separated into 5 subgenera as *Artemisia* Less., *Absinthium* (Mill.) Less., *Dracunculus* (Bess.) Rydb., *Seriphidium* (Bess.) Rouy., and *Tradentatae* (Rydberg) McArthur (Kurşat et al., 2015; Valles &McArthur, 2001). Most of *Artemisia* species have economic importance as therapeutics, foodstuff, fodder, esthetics and soil binders in destructive habitats; some taxa are poisonous or allergenic and some others are noxious weeds, which can badly affect crops (Tan *et al.* 1998, Hayat *et al.* 2009). Many *Artemisia* and *Seriphidium* speacies are used by the folk medicine (Nofal et al., 2009; Amin et al., 2019; Zhang et al., 2019; Zhang et al., 2018).

Many papers devoted anatomical (Janaćković et al., 2019; Rodica&Broască, 2012; Hussain et al., 2019; Abderabbi et al., 2018) and biochemical (Asilbekova et al., 2012; Velikorodov et al., 2011; Bodoev et al., 2000; Gilani et al., 2010) studies of *Seriphidium* (formely *Artemisia*) species have been published for the last few decades.

Janaćković et al. (2019) carried out the anatomical analysis of vegetative organs of five Serbia flora *Artemisia* L. (Anthemideae, Asteraceae) species (*Artemisia campestris* L., *A. absinthium* L., *A. arborescens* L., *A. judaica* L. and *A. herba-alba* Asso). Bercu & Broască (2012) studied the anatomical features of the Artemisia alba subsp. saxsatilis (Will.) P. Four. in Romania. Hussain et al. (2019) examined the anatomical characteristics of 13 species of Artemisia of the region of Pakistan. Abderabbi et al. (2018) studied morphological and anatomical parameters of the leaf and variations of Artemisia herba-alba Assopopulation in a steppe zone of western Algeria.

Velikorodov et al. (2011), Bodoev et al.(2000) investigated the main essential oil components of Artemisia lerchiana Web. and Artemisia santonica L. Gilani et al. (2010) studied the Seriphidium kurramense (Qazilb.) Y.R.Ling in Pakistan. Sefidkon et al. (2002) identified the essential oil componentsof 3 types of Artemisia spp. in Iran. Asilbekovaet al. (2012) identified the main essential oil components of Artemisia heptapotamica Poljak (now Seriphidium heptapotamicum Poljak).

The essential oil content of S.heptapotamicum (Poljak) Ling & Y.R. Ling have been studied by Asilbekova et al. (2012). But, the anatomy of S.heptapotamicum have not been studied.

The aim of this study is to:

determine root, stem and leaf anatomy of Seriphidium heptapotamicum;

identify essential oil composition of Seriphidium heptapotamicum.

Materials and methods

The samples of *Seriphidium heptapotamicum* (Poljak) Ling & Y.R. Ling, Asteraceae, were collected from Altyn- Emel National Natural Park,Kazakhstan (Figure 1), on September 29, 2019 (43°57’443” N and 079°00’416” E,652 m., Aksoy 3118). The plant species were identified by Prof. Dr. Ahmet AKSOY (Turkey) and candidate of biological sciences Bakhytzhamal Sultanova (Kazakhstan).

Figure 1 – Map of the Altyn- Emel National Park in Kazakhstan

Seriphidium heptapotamicum is herbs, perennial, 20-35 (-40) cm tall, with a thick rootstock, densely gray arachnoid tomentose, later partly glabrescent. Lower and middle stem leaves are petiole short; leaf blade is oblong-ovate or ovate-elliptic, 2-2.5 × 1-1.5 cm, 2-pinnatisect (1 or 2 – pinnatisect in middle leaves); segments are 3 or 4 (or 5) pairs; lobules linear is 3-5 × 0.3-0.5 mm, acute apically. Upper leaves and leaflike bracts are pinnatisect or entire. Synflorescence a is somewhat broad and elongated panicle. Involucre is oblong or ovoid, 1.5-2.5 mm in diam.; phyllaries sparsely arachnoid are pubescent (Figure 2 a) (Ling et al., 1988).

Anatomical studies were carried out on plant material that was found in a mixture of alcohol (70%). Sections of root, stem and leaves were used through paraffin method, each sectionwas taken by microtome and stained with safranin and fast-green (Johansen, 1944). Further anatomical investigation was carried out under a light microscope and photographs were taken by Leica DM750 research microscope.

Concerning the biochemical studies, air-dried aerial parts of the plant material underwent hydrodistillation for 3 h using a Clevenger type apparatus. The essential oils were analyzed by GC/MS using a Agilent 7890A GC-MSD system according to the literature (Shaimerdenova *et al.* 2018). An Innowax FSC column (60 m × 0.25 mm L, with 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, then kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Alkanes were used as reference points in the calculation of relative retention indices (RRI). Split ratio was adjusted at 40:1. The injector temperature was at 250°C. MS were taken at 70 eV. Mass range was from 35 to 450 m/z. Library search was carried out using the Wiley GC/MS Library and the TBAM Library of Essential Oil Constituents. Relative percentage amounts were calculated from TIC by a computer.

Results

This paper presents an anatomical study of the vegetative organs and the composition of the essential oil of Kazakhstan *S.heptapotamicum*.

Anatomical characteristics

When studying the anatomical structure of the vegetative organs of *S.heptapotamicum*, the following microdiagnostic signs were established (Figure 2 a-d and Table 1).

Root: – The largest average size of roots of *S.heptapotamicum*: epidermis (27.3±7.79 µm), cortex (36.32±10.68µm), phloem (16.08±9.83µm), cambium (20.08±6.72 µm), xylem (17.46µm), pith (11.04 µm). The outer layer of the primary cortex, the exoderm, consists of tightly closed polygonal cells, the walls of which are subsequently corked and perform a protective function. Then the main parenchyma (mesoderm) is located, which makes up the main mass of the primary cortex. Between xylem and phloem there is a wide cambial zone. Endodermis is not clearly seen (Table 1, Figure 2 b).

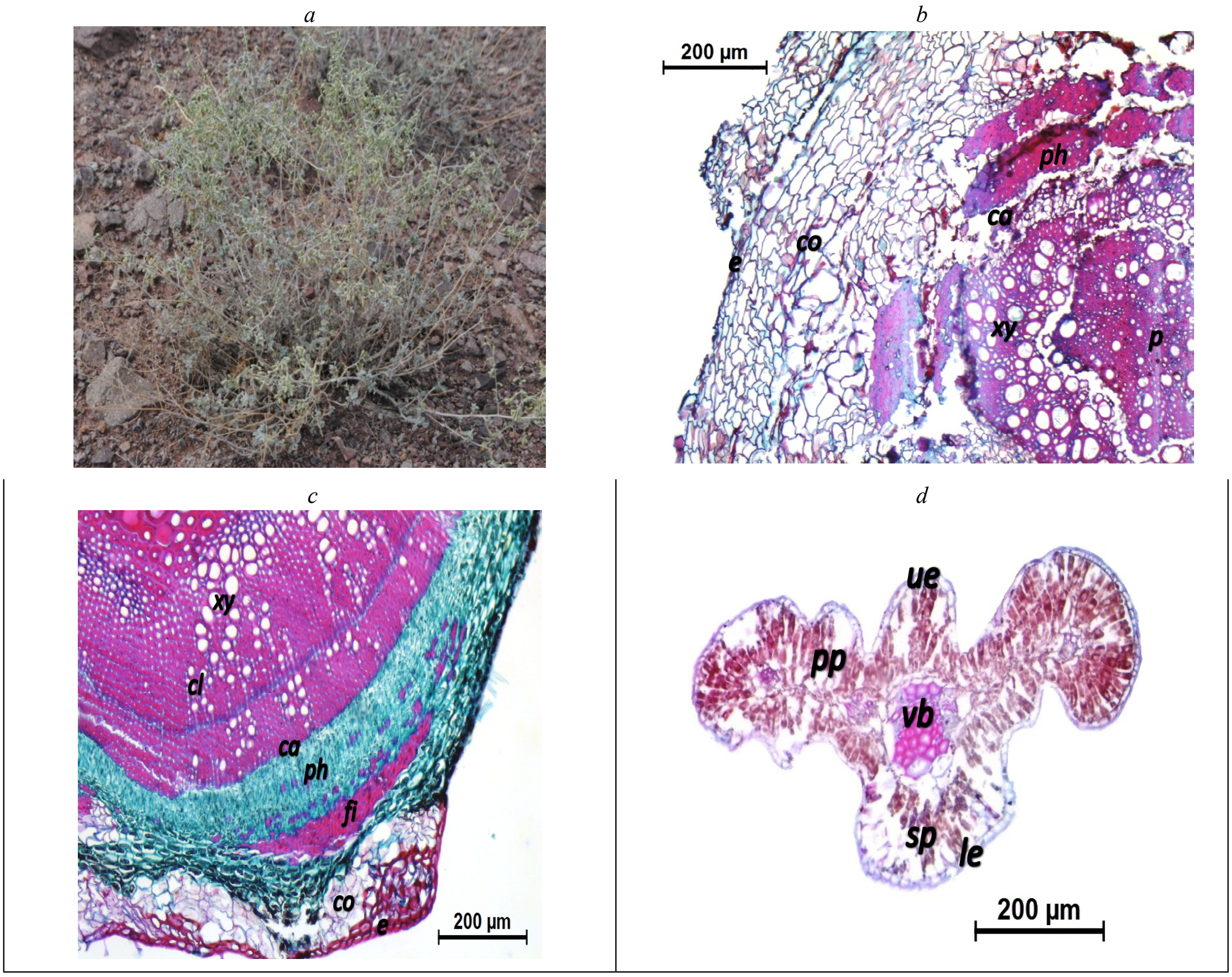


Figura 2 – *Seriphidium heptapotamicum* a. General view. b. Cross-section of root. c. Cross-section of stem. d. Cross-section of leaf (p: pith, co: cortex, ca: cambium, cl: chlorenchyma, fi-fiber, x: xylem, ph: phloem, e: epidermis, ue: upper epidermis, le: lower epidermis, pp: palisade parenchyma, sp: spongy parenchyma, vb: vascular bundle).

Table 1 – Anatomical measurements of *Seriphidium heptapotamicum.*

	Width (µm)				Length (µm)			
	Min. – Max.		Avr. ± Sd		Min. – Max.		Avr. ± Sd	
Root								
Epidermis cell	4.97	13.77	7.79	1.95	14.53	45.31	27.3	6.8
Cortex	3.93	24.63	10.11	3.5	13.33	52.24	28.64	8.2
Phloem	3.63	36.24	9.83	6.55	5.42	47.75	16.08	7.74
Cambium	4.23	16.6	6.72	2.28	12.25	34.52	20.08	4.39
Ksilem (diameter)	2.79	38.24	12.35	9.1				
Pith cell (diameter)	3.42	30.9	9.9	6.25				
Stem								
Epidermis cell	5.88	12.07	8.22	1.35	11.6	39.65	21.56	6.16
Cortex	5.51	31.22	12.23	4.13	15.49	53.42	29.6	7.66
Sclerenchyma	4.3	12.77	8.4	5.88	13.66	42.47	24.29	5.88
Perivascular fibers	5.91	14.1	8.95	1.63	10.45	28.83	17.48	3.26
Phloem	2.92	9.73	6.5	1.3	7.15	21.01	13.22	2.4
Ksilem (diameter)	3.49	29.7	15.03	6.69				
Pith cell (diameter)	4.83	46.99	14.07	8.04				
Leaf								
Epidermis cell	4.73	12.36	7.49	1.61	7.08	48.68	17.26	8.2
palisade parenchyma	4.48	11.06	7.7	1.46	14.35	46.03	27.28	6.43
spongy parenchyma	4.31	11.19	7.41	1.34	8.61	31.77	16.01	3.71
Sclerenchyma	4.84	15.33	9.13	2.72	11.05	49.11	23.59	7.85
vascular bundle (diameter)	5.22	20.9	12.18	3.97				
Min: minimum, Max: maximum, Avr: average, Sd: standard deviation.								

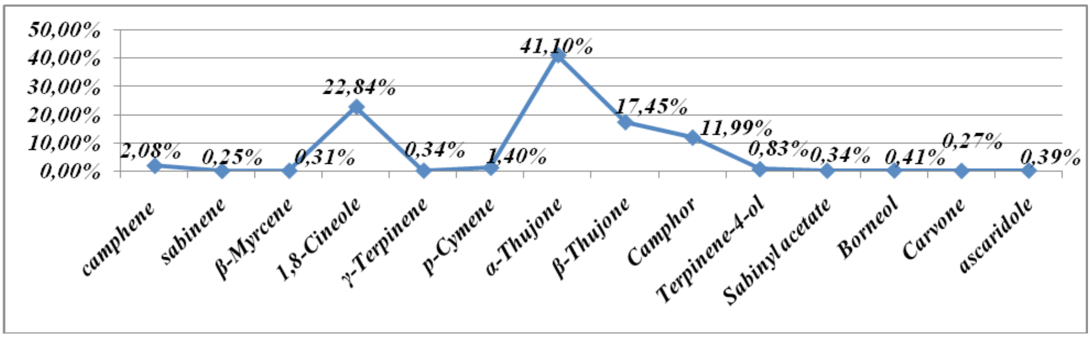
Stem: – The largest average size in stems of *S. heptapotamicum*: epidermis (21.56±8.22µm), cortex (29.6±12.23µm), sclerenchyma (24.97±8.58µm), perivascular fibers (17.48±8.95 µm), phloem (13.22±6.50µm), xylem (15.03µm), pith (28.93 µm). The epidermis is composed of single-row rectangular-like cells at the outermost layer. Beneath the epiderm, there is a cortex layer composed of 5-6 rows of parenchymatic cells. Right under this layer, there are 8–10 rows of well-developed chlorenchyma layer. Between parenchymal cells and sclerenchyma perivascular leaves are located. Fragmented epidermis cells are all covered with a thin cuticle layer at the outermost layer. The stele is represented by the phloem, poorly developed, protected in periphloemic groups of sclerenchymatous cells. The xylem is more developed than the phloem (Table 1, Figure 2 c).

Leaf: – The leaf cross-section clearly shows the 3 basic parts: epidermis, mesophyll tissue and vascular system. Mesophyll tissue is composed of two types of cells as palisade and sponge parenchyma. After the cuticle, it is possible to observe a single layer of regular and rectangular epidermal cells on both the upper and lower sides. There was no significant difference between the lower epidermis and upper epidermis cells. Beneath both the upper and lower epidermis, there is a palisade parenchyma which has two layers containing a large number of chloroplasts (Table 1, Figure 2 d).

Biochemical characteristics

Yields of essential oils are 2.4 % for *S.heptapotamicum*. The main components of *S.heptapotamicum* are essential oil were camphene – 2.08%, sabinene-0.25%, β-myrcene-0.31%, 1,8-cineole-22.84%, γ-terpinene-0.34%, p-cymene-1.40%, α-thujone-41.10%, β-thujone-17.45%, camphor-11.99%, terpinene-4-ol-0.83%, sabinyl acetate-0.34%, borneol-0.41%, carvone-0.27% and ascaridole-0.39%. The histogram displayes these components a as follows (Table 2).

Table 2 – Chemical compositions of *Seriphidium heptapotamicum* essential oils from Kazakhstan



Our study showed that the main constituents were α-thujone (41.10%), 1,8-cineole (22.84%), β-thujone (17.45%), and camphor (11.99%) for *S.heptapotamicum* essential oil. Other compounds were found in minor amounts (% 0.25- 2.08).

Discussion

The majority of studied rare *Artemisia* species including *S. heptapotamicum* have not been researched by other researchers for many years because of their foliar anatomical attributes.

Janaćković et al. (2019) described ‚a secondary anatomical structure, a well-developed xylem, a multilayer exodermisas in the cross section of the root using anatomical analysis of vegetative organs of five *Artemisia* L.(Anthemideae, Asteraceae) species (*Artemisia campestris* L., *A. absinthium* L., *A. arborescens* L., *A. judaica* L. and A. *herba-alba* Asso (now *Seriphidium herba-alba* (Asso) Soják) Our studies showed that secondary tissues were not revealed and a single-layer exaderms was covered (Fig. 2 b).The cross-sectional stems had a secondary structure due to the activity of the cambium, especially in the xylem region and xylem was more developed than phloem like in the *Artemisia alba* subsp. saxsatilis (Will.) P. Four (now *Artemisia alba* Turra) studied by by Bercu and Broaske (2012). Our study confirms this conclusion (Fig. 2c).

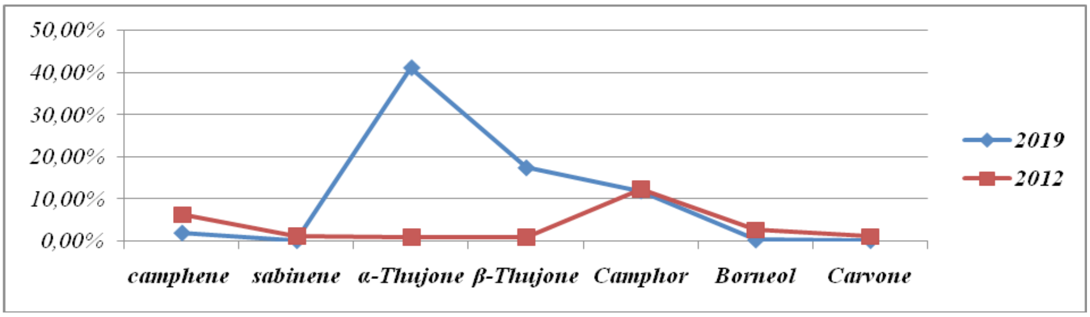
Hussain et al. (2019) have been examined the anatomical characteristics 13 species of *Artemisia* of the region of Pakistan and found out that *A. annua* L., *A. chamaemelifolia* Vill., *A.tournefortiana* Reichenb. (now *Artemisia biennis* Willd.), *A. verlotiorum* Lamotte, *A. indica* Willd., *A. Chinensis* L. (*Crossostephium chinense* Makino), *A. austriaca* Jacq., *A. gmelinii* Web., *A.vulgaris* L. and *A. dubia* Wall. ex Bess. showed irregular epidermal cells shape with wavy walls. *A. herba-alba* showed elongated shape of cells with smooth walls. *A. argyi* Levl.&Vaniot. and *A.montana* Pamp. showed polygonal shape with smooth walls. The epidermis of vegetative organs in our work consisted of single-row, dense, rectangular cells in the outer layer (Fig. 2 b-d). Abderabbi et al. (2018) have found that the chlorophyll in parenchyma is located on the periphery of leaf structure and the water reserve parenchyma is locateddeeper nearby the vascular bundles and have identified the main mechanisms for adaptation to drought. Our studies, revealed that the palisade and spongy parenchyma, which are located chloroplasts, matured well in *S.heptapotamicum* (Fig. 2 d).

In many species of *Artemisia lerchiana* Web. (formely *Seriphidium lerchianum* (Weber) Poljakov), *Artemisia santonica* L. (now *Seriphidium caerulescens* (L.) Soják), the main component of the essential oil is camphor (33-74%) (Velikorodov et al., 2011, Bodoev et al., 2000). Gilani et al. (2010) studied the *Seriphidium kurramense* (Qazilb.) Y.R.Ling (formely *Artemisia kurramensis* Qazilb.) in Pakistan and it was found that main component of essential oil consisted of α-thujone (26.0 – 73.4 %), β-thujone (3.14 – 49.3 %), 1,8-cineole (10.2 – 22.3 %) and camphor (0 – 26.3 %).

Essential oil from Iran was *A.aucheri* Boiss. (now *Seriphidium aucheri* (Boiss.) Ling &Y.R.Ling), *A. santolina* Schrenk (now *Seriphidium santolinum* (Schrenk) Poljakov), *A.sieberi* Bess. (now *Seriphidium sieberi* (Bess.) K.Bremer&Humphries ex Y.R.Ling). Twenty-six compounds were identified in the oil of *A.aucheri*, representing more than 79.6% of the oil. The main components of this oil were verbenone (21.5%), camphor (21.0%), 1,8-cineole (8.3%), trans-verbenol (8.1%) and p-cymene (3.5%). Thirty-nine compounds were identified in the oil of *A.santolina*, representing 89.6% of the oil. The main components of this oil were neryl acetate (13.4%), bornyl acetate (10.9%), trans-verbenol (9.9%), lavan-dulol (8.8%), linalool (6.9%), 1,8-cineole (6.5%) andgeranyl acetate (3.6%) (Sefidkon et al., 2002).

Asilbekova et al. (2012) previously reported cineole (41.5%), camphor (25.6%), camphene (6.4%), p-cymene (4.9%) and ketone (4.8%) as main constituents in the essential oil of *Seriphidium heptapotamicum*. When comparing data obtained in 2012 and 2019, the following table appears (Table 3). As we can see from the comparison table of the component composition of *A. sieberi* and *S.heptapotamicum*, the following components were similar sabine, camphor, borneol, carvone.

Table 3 – The comparison of essential oils content of two population of *S.heptapotamicum* in Kazakhstan



Forty compounds were identified in the oil of *A.sieberi*, representing more than 98.1% of the oil. The main components of this oil were camphor (49.3%), 1,8-cineole (11.1%), bornyl acetate (5.8%) and nerylacetate (4.3%) (Sefidkon *et al*.. 2002).

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