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EXPERIMENTAL PAPER

Ultrastructure of leaf epidermis in some species of *Achillea* (*Asteraceae*)

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Summary

Introduction: *Achillea millefolium* L. is used in official medicine of many countries. One of important diagnostic features of *A. millefolium* raw material is the ultrastructure of the leaf epidermis, which can be used for distinguishing *A. millefolium* from closely related species.

Objective: The purpose of this article is to present the results of an original study of the leaf surface ultrastructure of six related species of *Achillea* and to identify additional diagnostic features.

Methods: From each taxon, 20 samples of middle cauline leaves were taken and analysed with light (LM) or scanning electron (SEM) microscopy.

Results: The studied species differ in the degree of sinuosity of anticlinal walls of basic epidermal cells and their projections, the type and presence of epicuticular wax, the type of relief of the leaf surface and the type of cuticle. On both surfaces of the leaf lamina, there is a complex pubescence formed by uniseriate non-glandular and biseriate glandular trichomes; their structure is similar, but the size and localization are sometimes different.

Conclusion: It is possible to clearly distinguish the raw materials of any of the studied *Achillea* species from other taxa only with the help of the whole complex of the analysed species-specific micromorphological features of the leaf epidermis. This study is aimed at facilitating the identification of yarrow raw materials and can be used in the future for the development of regulatory documents for this herbal medicinal material, at least within the limits of the territory of Ukraine and adjacent territories.

Key words: ***Achillea, Ukraine, trichomes, leaf epidermis, ultrastructure***

Słowa kluczowe: ***Achillea, Ukraina, trichomy, naskórek liścia, ultrastruktura***

INTRODUCTION

The genus *Achillea* is represented in the flora of Ukraine by 27 taxa, according to the recent nomenclatural overview [1]. Views of researchers regarding the species status of some representatives of the genus remain debatable, because some of these taxa have only a few morphological features that can be used as diagnostic ones. In particular, in some databases *Achillea pannonica* Scheele is listed as a synonym of *A. seidlii* J.Presl & C.Presl [2], or *A. stricta* Schleich. ex Gremli is treated as a subspecies *A. distans* subsp. *stricta* (Gremli) Janch. [3-5]. The micromorphological features of the epidermis of these taxa in this article are analysed in the context of their conventional recognition as separate species.

The biologically active compounds of the genus *Achillea* have been identified for 14 species of the Ukrainian flora, and they are used or can be used as medicinal plants [6, 7]. The main biologically active substances of these *Achillea* species are volatile oils (sesquiterpenes and monoterpenes), flavonoids (as cynaroside, cosmosiin, luteolin, apigenin etc.), lignans, amino acid derivatives, fatty acids, and other components [6, 8, 9]. Species of *Achillea* are widely used in the official and traditional herbal medicines of various countries as hemostatic, analgesic, wound-healing, antibacterial, antiviral, choleretic, diuretic, antispasmodic, antipyretic, and anti-inflammatory agents. Many of these therapeutic properties have been confirmed by modern medical research [9]. According to the State Pharmacopoeia of Ukraine, the raw material of only one wild species of yarrow, *A. millefolium* L. (*Millefolii herba*), is standardized and allowed for the use in official medicine [10]. The raw material of other species contains biologically active substances similar to those of *A. millefolium*, but at present they are not standardized, so cannot be used in official medicine. However, the geographic ranges for most of these species overlap, and there are known cases of collecting of other neighbouring or co-occurring species. Such mixed raw material may erroneously get into production of medicines at pharmaceutical companies. Therefore, the identification of species-specific morphological features of these taxa is necessary for the authentication of raw materials of the most common *Achillea* species occurring in Ukraine and adjacent countries of Eastern and Central Europe.

Most of the species of the genus *Achillea* are characterized by their significant variability of macro- and micromorphological characters, that are genetically and ecologically determined [11–14], so there are difficulties in distinguishing species of the genus both for taxonomic purposes and for the identifications of medicinal raw materials. Literature reviews show that most of anatomical and morphological studies were devoted to *A. millefolium*, even if the species is accepted in a rather wide sense (*sensu lato*). The characteristics of macro- and micromorphological features of raw material of this species are given in pharmacopoeias [10, 15, 16] and other pharmaceutical publications and documents [17, 18].

Fragmentary studies of the anatomical structure of vegetative and generative organs of some yarrow species are known [12, 19–22]. In recent years, there has been growing interest to *Achillea* species of Ukraine as medicinal plants, and the morphological and anatomical features of a number of species (*A. ochroleuca* Ehrh., *A. micranthoides* Klokov, *A. stricta*, *A. carpatica* Blocki ex Dubovik, and *A. distans* Waldst. & Kit. ex Willd.) have been investigated [23–27].

The identification of yarrow raw materials is based on the analysis of morphological and anatomical features of vegetative (stems, leaves) and generative (inflorescences, flowers) organs. Features of the ultrastructure of the leaf surface are important diagnostic criteria for the differentiation of taxa and for detecting impurities in medicinal raw materials [21, 22].

The purpose of our research was to identify the features of ultrastructure of the leaf epidermis of six species of the genus *Achillea* most commonly occurring in Ukraine and adjacent countries of Eastern and Central Europe, and to identify their additional diagnostic features.

MATERIALS AND METHODS

The objects of the study were six related species of the genus *Achillea: A. millefolium, A. collina* Becker ex Rchb., *A. nobilis* L., *A. pannonica, A. setacea* Waldst. & Kit. and *A. stricta.* The nomenclature of the studied species of the genus is given according to Vascular Plants of Ukraine, a nomenclatural check-list [1].

Herbarium specimens of the studied species of genus *Achillea* were taken from the National Herbarium of Ukraine (herbarium of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine – KW) and fresh samples of *Achillea* were collected from different localities of Ukraine during the period from July 2019 to August 2020 and identified according to the reference collections at KW (tab. 1).

In order to study the ultrastructure of the surface of sheet leaves by scanning, electron microscope was used. The leaves were dehydrated in silica gel for 2–3 days. Dried samples of leaves for scanning electron microscope research (SEM) (JSM-6060 LA; JEOL) were processed according to standard method: dehydrated fragments of leaves were fixed on brass tables and sprayed with a thin layer of a mixture of gold and platinum in the vacuum chamber.

For studies of the epidermal tissue of leaves in the paradermal plane by method of light microscope, the objects were fixed in a mixture of 70% ethanol–acetic acid–formalin followed by tissue maceration. Maceration of plant tissues was carried out in a macerating solution (hydrogen peroxide–glacial acetic acid-water), pieces of leaf lamina were kept at 25°C for 3 to 7 days to dissecting the upper (adaxial) and lower (abaxial) epidermis. Two epidermal layers were stripped and stained with 1% hematoxylin solution. Slides were observed by using Olympus CX23 light microscope (LM) with a Levenhuk M 1000 Plus digital camera and Levenhuk Lite software.

The quantitative parameters (length and width of stomata, length of glandular trichomes (glands) along the major and minor axes, length of distal cell of non-glandular trichomes (hairs) was calculated using Levenhuk Lite and AxioVision 4.8 software.
The sample size for calculating the density of glandular and non-glandular trichomes was 20 for the adaxial and abaxial epidermis of each species. To determine the morphometric parameters of glandular and non-glandular trichomes and stomata (the length of the distal cell of the non-glandular trichome, the length of the glandular trichome along the major and minor axes, the length and width of the stomata) – in a 50-fold repetition were made. Data analysis was carried out in the STATISTICA 10.0 program, the results were presented as the mean ± confidence interval with an accepted level of significance ($p<0.05$).

We determined the type of epicuticular wax based on W. Barthlott's work [28, 29], the types of relief are described in C. Chakrabarty's and P. Mukherjee's work [30]. To describe outlines of cells and their projection onto the plane we used S. Zakharevich's terminology [31]. The types of cuticle are determined based on C. Jeffree's work [32].

Ethical approval: The research conducted is not related to either human or animal use.

RESULTS AND DISCUSSION

The observed morphological characters of the epidermis of model *Achillea* species are listed in Table 2, 3 and illustrated in figures 1–5. Generally, the main diagnostic descriptions of the leaf epidermis of *Achillea* were described as follows:

Adaxial surface of the leaf lamina. The type of relief of the adaxial epidermis of the leaf lamina of almost all studied species (except *A. pannonica*) is colliculate-spinous (periclinal walls are unevenly convex, anticlinal walls of epidermal cells are lower than periclinal ones) (tab. 2, fig. 1a). The adaxial surface of the leaf lamina *A. pannonica* is colliculate (periclinal walls are evenly convex, anticlinal walls of epidermal cells are lower than periclinal ones) (fig. 1b). Cuticle of most species is well expressed in all studied specimens, but it is not uniform in structure of some species (tab. 2). Thus, cuticle of the main epidermal cells of *A. stricta* is smooth, and the surrounding stomatal cells have a striated cuticle (fig. 1c). In studied specimens of *A. setacea*, the smooth (fig. 1d) and striate cuticle of epidermal cells were found. In other four species, the cuticle is striated (tab. 3, fig. 1e). The films and plates of epicuticular wax are present at the adaxial epidermis of *A. stricta* and *A. pannonica* (fig. 1f, g); while in *A. setacea* and *A. nobilis* only wax films were found (fig. 1h, i) and it is absent at the adaxial surface of *A. collina* and *A. millefolium* (tab. 2). The projections of epidermal cells of most species above the mesophyll are flattened (according to Zakharevich [31], the cell projection is considered to be flattened with complex cell outlines (the lines of the walls of each side are different in length and curvature of the arc), under the condition that the longer axis is

Table 1.

Specimens of the *Achillea* species analyzed in the study

Species	Locality	Collecting Date	Collectors, Herbarium code
Achillea millefolium	Kyiv region, Makariv district, near the village of Mykolaivka, the abandoned fields	15.06.2012	Minarchenko V.M., Solomakha T.D., Tymchenko I.A., KW 155683
	Poltava region, Lokhvytsya district, near the village of Pisky, meadow	31.07.2013	Olshansky I.G., Dvirna T.S., KW № 155693
	Kyiv region, Obukhiv district, near the village of Kozyn, the floodplain meadow of Kozynka river	14.07.2019	Tymchenko I.A., KW 155684
A.collina	Kyiv region, Makariv district, near the village of Mykolaivka, weakly turfed dry sandy slopes	15.07.2000	Minarchenko V.M., KW 155682
	Chernigov region, Novgorod-Seversky district, village Putivsk, meadow on the banks of the Desna	07.07.1982	Krytska L.I., KW 017786
	Chernigiv region, Talalaiv district, village Lavirkove, on the side of the road	5.09.1978	Krytska L.I., Akopyants N.S., KW 017779
A. nobilis	The city of Odessa, dry slopes over the sea below the botanical garden	4.08.2004	Tsarenko O.M., Krytska L.I., KW 041299
	Kherson region, Beryslav district, near the village of Kochkarivka, sandy-rocky slopes	15.06.2013	Solomakha T.D., KW 155685
	Dnipro region, Synelnykove district, near the village of Pavlivka, the slopes on the bank of the Kamianske reservoir	11.08.2020	Glushchenko L.A., KW 155686
A. pannonica	Crimean region, Arabat arrow, sand-rocky steppe	07.03.1976	Androschuk A.F., Krytskaya L.I., Ilyinskaya A.F., KW 00105407
	Kirovograd region, Svetlovodsk, sands on the bank of the Kremenchug reservoir	09.08.1978	Krytskaya L.I., Akopyants N.S., KW 001055406
	Kherson region, Beryslav district, near the village of Kochkarivka, sandy-rocky slopes	15.06.2013	Solomakha T.D., KW 155687
A. setacea	Mykolaiv region, Mykolaiv district, near the village of M. Korenikha, slopes on the right bank of the Bug estuary	25.07.1976.	Krytska L.I., Ilyinska A.P., Androschuk A.F., KW 019576
	The city of Kyiv, Poznyaky, the roadsides	03.07.2019	Minarchenko V.M., KW 155689
	The city of Odessa, the slope below the botanical garden	12.07.2019	Tsarenko O.M., KW 155688
A. stricta	Transcarpathian region, Tyachiv district, near the of village Ruska Mokra	13.08.2009	Minarchenko V.M., KW 155690
	Ivano-Frankivsk region, Yaremche district, near the village of Yablunytsya, Yablunytskyi passage, 940 m a. s. l.	6.07.2019	Minarchenko V.M., KW 155691
	Transcarpathian region, Rakhiv district, Carpathian Biosphere Reserve	24.07.2019	Klimovych N.B., KW 155692

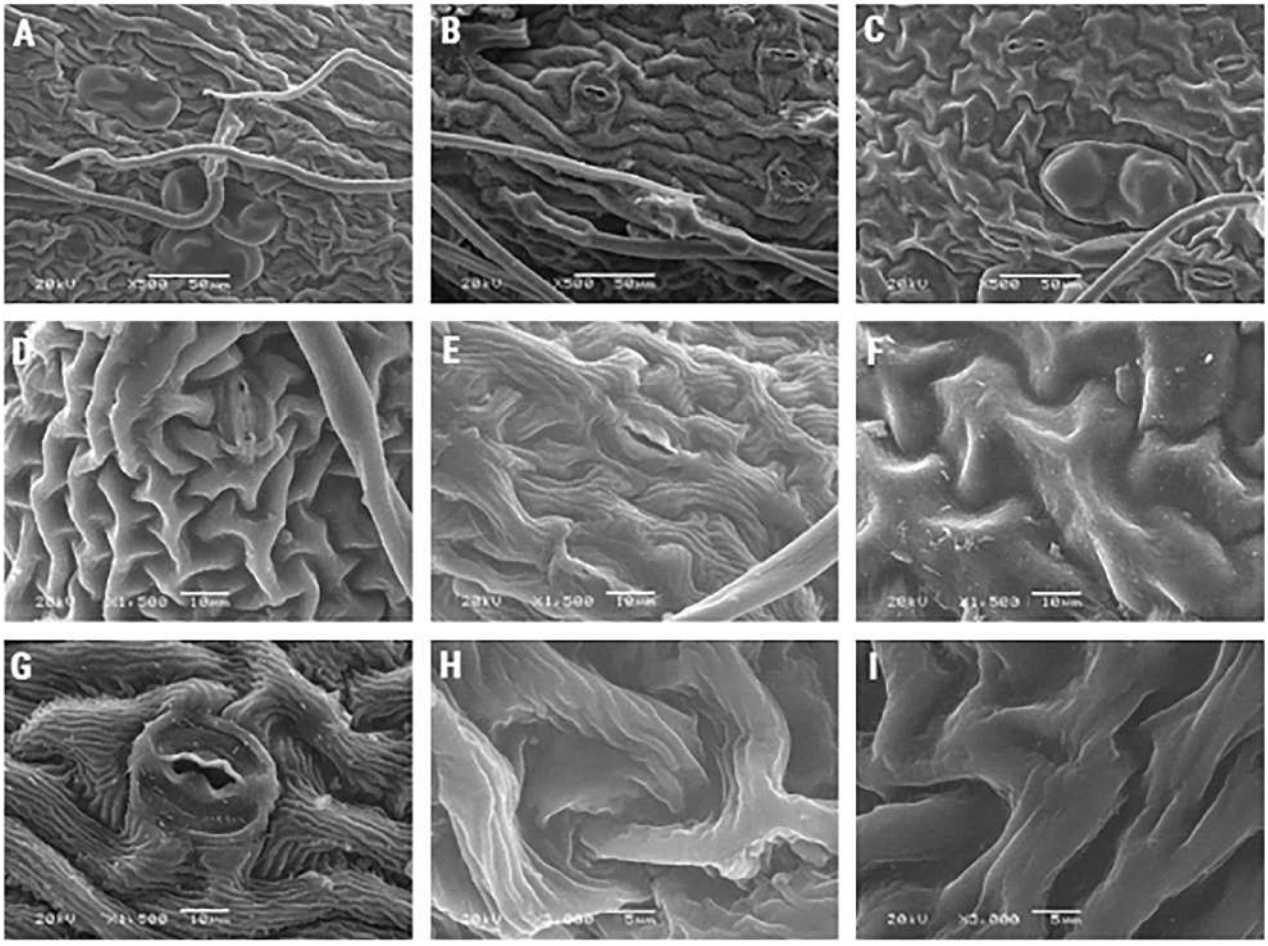

Figure 1.

Variation of type of relief, cuticle and epicuticular wax of adaxial surface of leaf lamina of some *Achillea* species (SEM) Type of relief: A – colliculate-spinous (*A. millefolium*), B – colliculate (*A. pannonica*); type of cuticle: C – striate cuticle of subsidiary cells and smooth cuticle of the main epidermal cells (*A. stricta*), D – smooth (*A. setacea*), E – striate (*A. nobilis*); type of epicuticular wax: F – films and plates (*A. stricta*), G – films and plates (*A. pannonica*), H – films (*A. nobilis*), I – films (*A. setacea*)

approximately equal to the transverse) (tab. 2, fig. 2a). In *A. setacea*, flattened and elongated epidermal cells were found (tab. 2, fig. 2b, c). The anticlinal walls of epidermal cells of the adaxial surface are characterized by varying degrees of sinuosity. The outlines of cells of *A. millefolium*, *A. pannonica* and *A. setacea* have round-sinuous outlines (fig. 2d), *A. collina* – slightly sinuous (fig. 2e), *A. nobilis* and *A. stricta* – sinuous (fig. 2f).

An important diagnostic feature of the epidermis of yarrow leaves is pubescence by non-glandular and glandular trichomes. In our study, it was established that the structure of glandular and non-glandular trichomes of the selected species is generally similar, but quantitative parameters, such as size of glandular and non-glandular trichomes and their density differ one from another. The data we obtained on the density and size of trichomes differ slightly from those previously published ones [12, 13] which can be related to environmental conditions.

The structure of non-glandular trichomes is similar in all studied species: they consist by 1–2 rounded or trapezoidal basal cells that have a well-defined striated cuticle, 4–7 isodiametric cells and a long distal cell (fig. 2m, n). The highest density of non-glandular trichomes was found on the adaxial surface of *A. pannonica* (64.53±1.34 tr./1 mm^2) (tab. 3, fig. 3d). This value is almost twice as small in *A. nobilis* (tab. 3, fig. 3c) and the density of non-glandular trichomes on the epidermis of *A. collina* and *A. setacea* is almost the same (tab. 3, fig. 3a, e). The adaxial epidermis of

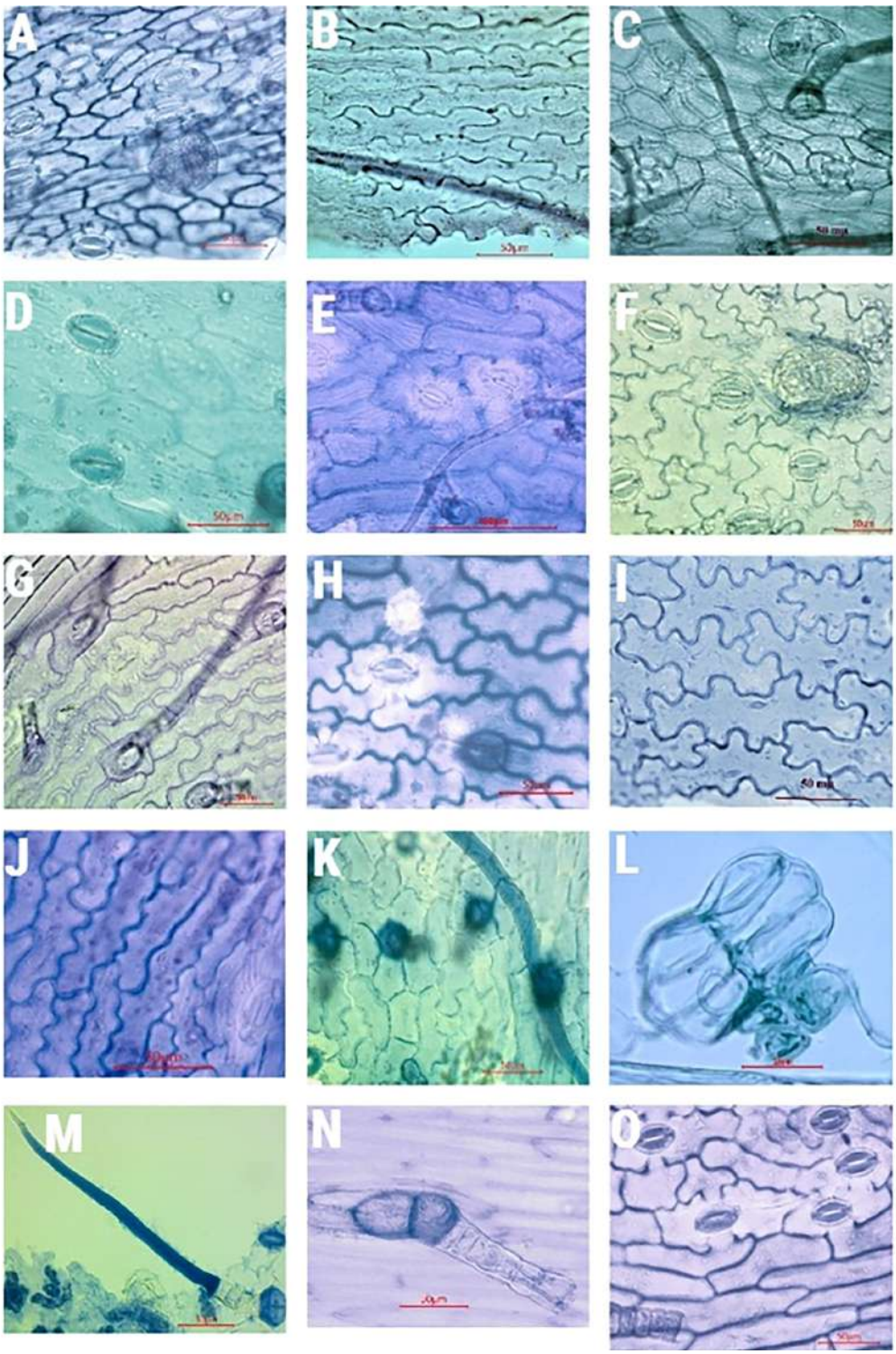

Figure 2.

Variation of projections and outlines of epidermal cells over the mesophyll of leaf lamina of some *Achillea* species, glandular and non-glandular trichome, stomata (LM)

Adaxial surface, projections of epidermal cells: A – flattened (*A. millefolium*), B – elongated (*A. setacea*), C – flattened (*A. setacea*); outlines of epidermal cells: D – round-winding (*A. pannonica),* E – slightly sinuous (*A. collina*), F – sinuous (*A. stricta*); abaxial surface, projections of epidermal cells: G – elongated (*A. setacea*), H – flattened and elongated (*A. collina*), outlines of epidermal cells: I – sinuous (*A. stricta*); J – finely sinuous (*A. millefolium*), K – round sinuous (*A. nobilis*); trichomes: L – glandular (*A. setacea)*, M – non-glandular (*A. nobilis*), N – base of non-glandular trichome (*A. stricta*); O – anomocytic stomata (*A. setacea*)

Table 2.

Peculiarities of the ultrastructure of the leaf epidermis of *Achillea* species

Name of species	*A. collina*	*A. millefolium*	*A. nobilis*	*A. pannonica*
Type of stomatal apparatus	anomocytic			
Adaxial surface				
Type of relief	colliculate-spinous			colliculate
Type of cuticle	striate			
Outlines of epidermal cells above mesophyll	slightly sinuous	round sinuous	sinuous a6o sinuous	round- sinuous
Projections of epidermal cells above mesophyll Polygonal irregular	flattened			
Epicuticular wax	absent		films	
Abaxial surface				
Type of relief	colliculate-spinous		colliculate	
Type of cuticle	striate			
Outlines of epidermal cells above mesophyll	sinuous	finely sinuous	round sinuous	
Projections of epidermal cells above mesophyll	flattened and elongated	elongated	flattened and elongated	elongated
Epicuticular wax	films and plates		films, plates, sometimes crusts	films and plates

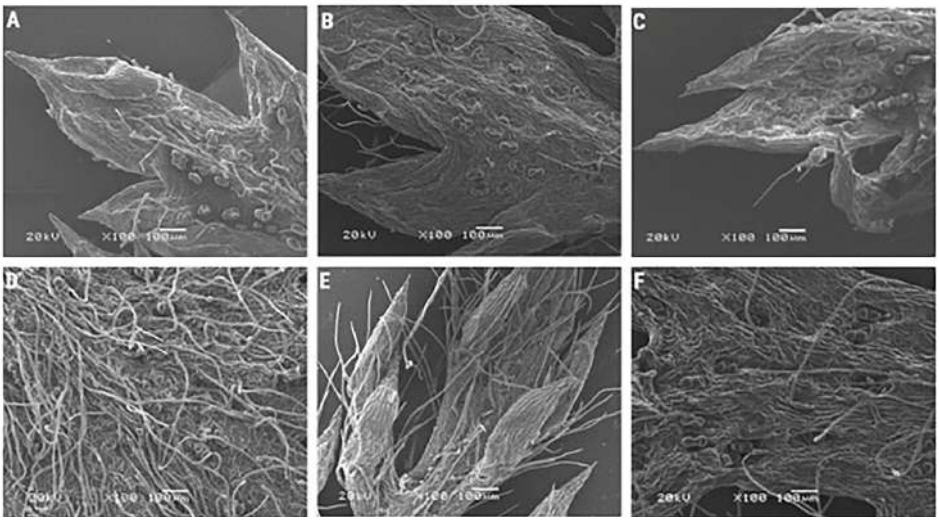

Figure 3.

Glandular and non-glandular trichomes of the adaxial surface of leaf lamina of *Achillea* species
A – *A. collina*, B – *A. millefolium*, C – *A. nobilis*, D – *A. pannonica*, E – *A. setacea*, F – *A. stricta*

A. setacea	*A. stricta*
colliculate-spinous	
from smooth to striate	
	sinuous
elongated and flattened	flattened
	films and plates
colliculate-spinous	
sinuous	
films	films, plates, sometimes crusts

A. millefolium and *A. stricta* is the least pubescent in our study (tab. 3, fig. 3b, f).

The length of the distal cell of non-glandular trichomes above the leaf mesophyll also has signs of species specificity. The longest terminal cells of non-glandular trichomes were found in *A. stricta*, they are slightly smaller in *A. pannonica* and the shortest in *A. collina* (tab. 3). The length of the distal cell of the trichomes located above the veins is significantly longer than above mesophyll in all studied species, except for *A. nobilis*, which has almost the same parameters of the trichomes on the adaxial surface (tab. 3).

Glandular trichomes of the investigated *Achillea* species are double-rowed, formed by 2 rows of 3–5 cells in each, forming a short stem and a head (fig. 1c, 2l). The glands are elliptical in horizontal projection and are oriented with their longer axis along the veins of the leaf (fig. 1a, 3a-f). The highest density of glands on the adaxial surface of the leaf was found in *A. nobilis* (tab. 3). According to the decrease of this indicator, the studied species can be placed in the following order: *A. nobilis*→ *A. collina*→ *A. pannonica*→ *A. setacea* → *A. stricta* → *A. millefolium*. The density of glands in all analysed specimens is significantly higher on the adaxial surface than on the abaxial surface (tab. 3). *A. setacea* has the smallest glandular trichomes, and *A. stricta* has the largest ones (tab. 3, fig. 2l, 3e, f).

All studied species of the genus *Achillea* are characterized by an amphistomatic leaf, stomata are located on both surfaces of the leaf. Stomata are

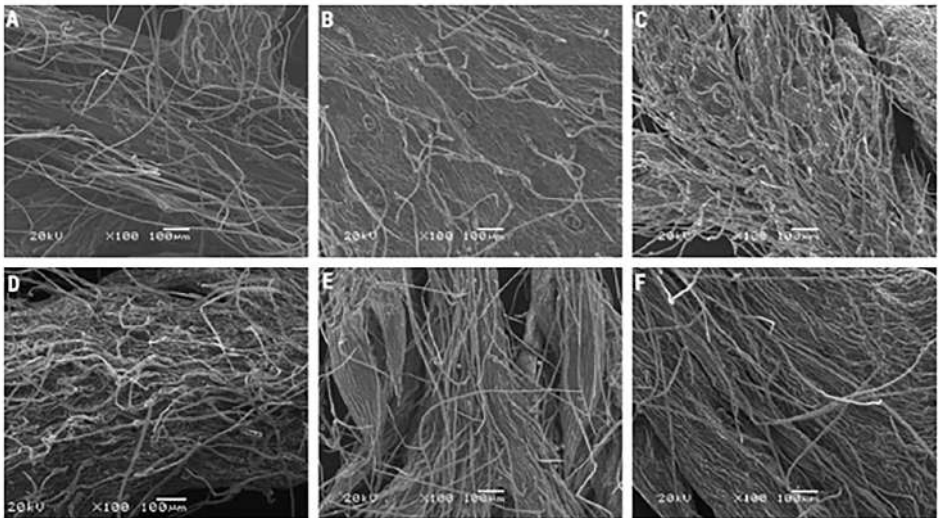

Figure 4.

The glandular and non-glandular trichomes of the abaxial surface of leaf lamina of some *Achillea* species
A – *A. collina*, B – *A. millefolium*, C – *A. nobilis*, D – *A. pannonica*, E – *A. setacea*, F – *A. stricta*

Table 3.

Quantitative data of the leaf surface characteristics of some *Achillea* species

Name of species	*A. collina*		*A. millefolium*		*A. nobilis*	
Epidermis	**adaxial**	**abaxial**	**adaxial**	**abaxial**	**adaxial**	**abaxial**
Glandular trichomes						
Number of trichomes per 1 mm^2	24.07±0.78	5.47±0.97	11.57±0.67	2.97±0.45	39.83±1.34	11.95±0.39
Ratio of trichome number on adaxial/ abaxial sides	4.40:1		3.90:1		3.33:1	
Length of trichomes along the major axis [μm]	68.12±2.09	63.90±2.76	73.20±1.99	63.98±2.13	73.5±3.01	73.1±3.89
Length of trichomes along the minor axis [μm]	42.70±2.01	38.50±1.34	41.2±2.31	36±1.92	36.4±1.26	31.5±1.65
Non-glandular trichomes						
Number of trichomes per 1 mm^2	23.16±1.34	60.29±2.12	9.94±0.34	22.07±0.34	30.12±1.56	84.14±2.56
Ratio of trichome number on abaxial/ adaxial sides	2.60:1		2.22:1		2.79:1	
Length of the distal cell of trichomes located above veins [μm]	216±5.12	275±6.89	439±9.87	576±10.4	217±13.09	549±12.9
length of the distal cell of trichomes located above mesophyll [μm]	133±2.60	179±2.39	259±3.83	337±8.78	215±8.35	410±7.96
Stomata						
Length [μm]	30.32 ±0.99	33.75±1.25	31.62±1.12	31.54±2.15	28.11±1.35	22.53±1.35
Width [μm]	21.25±0.56	21.47±0.67	20.51±1.02	19.46±0.98	21.28±0.87	19.52±079

anomocytic, from rounded to oval (ratio of stomata length to width varies within 1.17–1.55: 1) (tab. 3, fig. 2a, c-h, o). Stomata with their longer axis oriented along the veins and are located at the same level as the basic epidermal cells (fig. 2o). It is known that the size of stomata in *Achillea* taxa significantly depends on the level of ploidy, stage of ontogenesis and environmental conditions [12, 13]. Therefore, their diagnostic role is insignificant. In our study, the largest stomata were found in *A. millefolium* and *A. stricta*, the smallest in *A. nobilis* and *A. setacea* (tab. 3).

Abaxial surface. The abaxial surface of the leaves of the studied yarrow species has a number of different features from the adaxial surface. In particular, in most species, the abaxial epidermis is more pubescent than the adaxial one, with the exception of *A. pannonica*, the leaves of which have almost the same pubescence on both sides (tab. 3, fig. 3d, 4d). Distal cells of non-glandular trichomes

A. pannonica		*A. setacea*		*A. stricta*	
adaxial	**abaxial**	**adaxial**	**abaxial**	**adaxial**	**abaxial**
21.05±1.09	3.04±0.12	18.31±0.99	6.36±0.87	14.03±0.45	2.10±0.32
6.92:1		2.88:1		6.68:1	
76.9±2.76	76.8±2.54	61.76±3.97	64.32±4.01	91.35±3.01	80±3.54
37.5±1.25	35.5±2.01	31.6±1.45	30.5±1.55	46.5±2.35	36±1.45
64.53±1.34	69.15±2.90	25.29±0.34	59.06±2.78	8.96±0.97	25.52±0.34
1.07:1		2.34:1		2.85:1	
654±10.3	667±10.36	625±9.98	675±8.34	703±10.20	987±15.90
415±10.32	450±5.65	230±5.12	295±7.35	430±2.54	615±3.15
30.18±1.43	30.46±2.60	24.53±1.87	25.68±3.12	33.72±1.34	34.5±2.01
24.9±1.02	21.45±1.23	17.44±1.08	18.65±0.23	24.05±1.67	22.45±1.56

of the abaxial epidermis are longer than those on the adaxial surface in all species (tab. 3). The ratio of the number of glandular trichomes on the adaxial/abaxial sides is also different, although in general, in all samples, the density of glands on the adaxial surface is 3-6 times higher than that on the abaxial surface (tab. 3, fig. 3a-f, 4a-f). The smallest density of glands is found on the abaxial epidermis of *A. stricta*, but they exceed those of other species in size (tab. 3).

The projections of epidermal cells of the abaxial surface of most studied species are elongated (fig. 2g), only cells of *A. nobilis, A. collina* have flattened and elongated projections (fig. 2h). Epidermal cells above the mesophyll of *A. collina, A. setacea* and *A. stricta* have sinuous outlines (fig. 2i), while in *A. millefolium* they are finely sinuous (fig. 2j), and in *A. nobilis* and *A. pannonica* round-sinuous (fig. 2k).

We did not find a clear difference in the size of the stomata on the adaxial/abaxial surface of the leaves

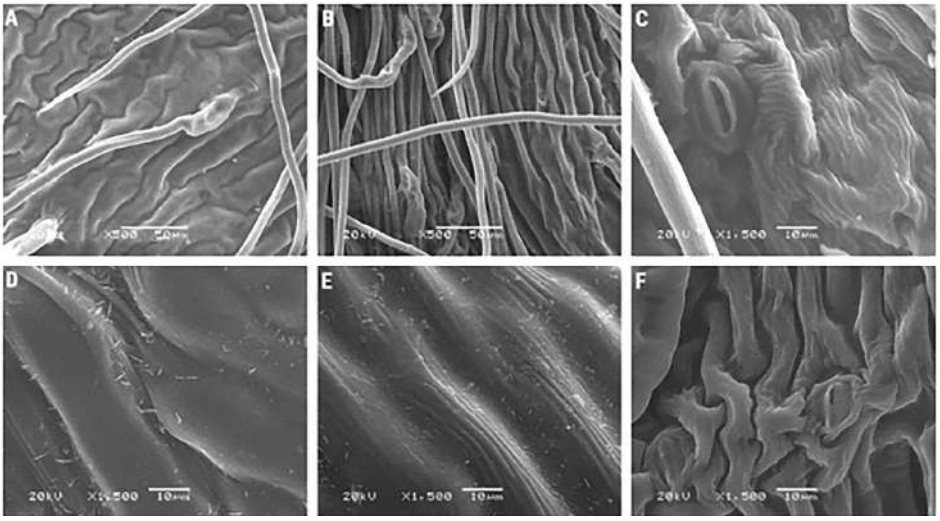

Figure 5.

Variation of type of relief, cuticle and epicuticular wax of abaxial surface of leaf lamina of some *Achillea* species (SEM) Type of relief: A – colliculate (*A. pannonica*), B – colliculate-spinous (*A. setacea*); type of cuticle: C – striate (*A. nobilis*); type of epicuticular wax: D – films and plates (*A. collina*), E – films, plates and crusts (*A. stricta*), F – films (*A. setacea*)

of the studied species (tab. 3). Only in *A. nobilis* the length of stomata on the abaxial epidermis is clearly shorter than that on the adaxial one. This may be related to environmental conditions, so it is impossible to assert the diagnostic value of this characteristic.

The relief of the abaxial surface of the *A. nobilis* and *A. pannonica* leaf lamina is colliculate (fig. 5a), in other species it is colliculate-spinous (fig. 5b). The cuticle of epidermal cells is striated and well-defined in all examined samples (fig. 5c). The epicuticular wax is better expressed on the abaxial surface of the leaf lamina of all studied species and represented by plates, films, sometimes crusts (tab. 2, fig. 5d-f).

CONCLUSIONS

With the general similarity of *Achillea* species, a number of species-specific features are inherent in the ultrastructure of the surface of leaves. Raw material (leaves) of any of the studied *Achillea* species can be clearly distinguished from other related species only through the use of the whole complex of species-specific micromorphological characteristics. Complex include the following features: the degree of tortuosity of the anticlinal walls of the main cells of the epidermis and their protrusions, the type and presence of epicuticular wax, type of relief of the leaf surface, type of cuticle, dimension of glandular trichomes, ratio of number on abaxial/adaxial sides glandular and non-glandular trichomes and the length of non-glandular trichomes distal cells. This study is aimed at facilitating the identification of *Achillea* species and might be further used for the development of standard on this herbal raw material.

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