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Chemotaxonomic and anatomical differences between some species of the genus Geranium L. of flora of Ukraine

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The chemotaxonomic and anatomical differences between some species of the genus Geranium L. of the flora of Ukraine have been discovered: *Geranium robertianum L.*, *Geranium sibiricum L.*, *Geranium sanguineum L. i Geranium macrorrhizum L.*

Keyword: Species of the genus Geranium L., Volatile compound, Chromatographymass-spectrometry method, Leaf anatomy.

1. Introduction

According to the literature, the genus Geranium plants contain a considerable amount of biologically active substances of different classes (tannins, flavonoids, hydroxycinnamic acids, anthocyanins, hydrocarbons, nitrogen-containing compounds, etc.) [1–4].

In the experiments in vivo and in vitro, they exhibit antibacterial, antiviral, anti-inflammatory, analgesic, antioxidant and anti-hypertensive activity, and, thus, have a potential therapeutic value.

Therefore the genus Geranium recently attracts attention of many researchers. In particular there are also described the studies of the anatomical structure in connection with the study of phylogenetic relationships and clarifying of taxonomy questions [5], the identification of adaptation to growth in different ecological conditions [6, 7].

The flora of Ukraine numbers 24 species of the genus Geranium L., especially in the Crimea are growing representatives of 17 species [8]. Plants of the genus Geranium L. very differ by morphological features, but they have a similar range of biologically active substances [9]. In the available literature the data concerning chemotaxonomic and anatomico-microscopic differences between different species of the genus Geranium L. of flora of Ukraine are absent. Therefore, such studies are relevant.

2. Aim of study:

The aim of this work was to establish the chemotaxonomic and anatomical features of four species of the genus Geranium L. of flora of Ukraine. As objects of study are selected four species of the genus Geranium L. of flora of Ukraine - *Geranium robertianum L.*, *Geranium sibiricum L.*, *Geranium sanguineum L.* and

Geranium macrorrhizum L. The raw material was gathered in phenophase of the mass flowering on experimental plots of Kyiv O.V. Fomin Botanical Garden and in wild form in the suburbs of Kyiv.

3. Materials and Methods

According to the findings of our previous research, plants of the genus Geranium L. are similar by chemical composition of the following groups of biologically active substances as flavonoids, procyanidins, gallo-ellagotannins [10, 11]. Therefore, we study chemotaxonomic characteristics for substances of a majority class of biologically active substances of the genus Geranium L., the volatile compounds, by gas chromatomas spectrometry method.

To the sample of dry material (0.5-5.0 g) was added an internal standard (tridecane) averaged 50 μg per sample. In a sample was added 10 ml of water and distilled off the volatiles compounds of samples with steam for 2 hours using a reflux air-cooled condenser. The adsorbed on the inner surface of reflux condenser, the volatiles substances after cooling of systems were washing with slow addition of 3 ml of especially pure pentane in a dry vial of 10ml capacity. The washings were concentrated with blowing (100 ml/min) of especially pure nitrogen to a residual volume of extract 10 μl that is fully selected with chromatographic syringe. The further concentration of the sample was carried out in the syringe to the volume 2 μl. The introduction of the sample into the chromatography column was performed using splitless, that is without a flow separation, which allows you to enter the sample without losing distribution and significantly (10-20 times) increase a sensitivity of method.

The rate of the sample introduction was 1.2 ml / min for 0.2 minutes. For studies was used the following chromatographic system: gas chromatograph Agilent Technologies 6890 with mass spectrometric detector 5973. The chromatographic column - DB- 5 capillary with an inner diameter of 0.25mm and a length of 30m. The rate of carrier gas (helium) 1.2 ml / min. The temperature of the heater input samples – 250 ºC. The temperature of thermostat is programmable from 50ºC to 320ºC at a rate 4dg/min.

To identify components was used the library mass spectra NIST05 and WILEY 2007 with a total of more than 470,000 spectra, combined with programs to identify AMDIS and NIST. For quantitative calculations was used the method of internal standard [12].

For anatomical studies were used the middle part of lamina and petiole of G. macrorrhizum L., G. sibiricum L., G. sanguineum L. and G. robertianum L. The samples were fixed by Chamberlain [13]. They were embedded in gelatin by the standard method [14] and were made leaf and petiole cross-sections (thickness of 10-15 microns) by the freezing microtome. The sections were stained with safranin. Also was performed the maceration of leaves to study the structures of epidermis of the adaxial and abaxial leaf surfaces. The structure of the epidermis was described following Zakharevich and Baranova [15]. The stomata index (SI) was found using the formula SI = NS / (NS + NE), where NS - the number of stomata per 1 mm2 of the epidermis surface, NE - epidermis cells quantity per 1 mm2 of the epidermis surface. The microscopic measurements were carried out using an eyepiece-micrometer on microscope XSP-146TR. The obtained data were statistically processed with the program Statistica 6 on $P \leq 0.05$ confidence level. The pictures were taken by the digital camera Canon Power Shot A630.

4. Results and Discussion

According to the obtained results concerning determination of the qualitative composition of the volatile compounds, species of the genus Geranium L. differ by composition and quantitative content of volatile substances, though they belong to the same genus. The identified volatile substances belong to different classes of biologically active substances: fatty acid esters, fatty acids, terpenes and terpenoids, aliphatic and alicyclic alcohols, aldehydes, ketones, aromatics, saturated aliphatic hydrocarbons, heterocycles. In grass of the studied species of the genus Geranium L. were identified 139 volatile compounds, including 2 fatty acid esters, 21 fatty

acids, 50 compounds – derivatives of terpenes and terpenoids, 17 compounds belonging to the class of aliphatic alcohols and ketones, 2 alicyclic ketones, 6 substances of aromatics, 1 alicyclic alcohol, 13 aliphatic alcohols, 22 alkanes, 1 nitrogen- and 2 oxygen-containing heterocycles.

The study allowed to determine specific volatile substances for elevated parts of each of the investigated species of the genus Geranium L., listed in Table 1.

Among all classes of volatile compounds (specific for each species and raw materials), we have chosen the substances related to terpenes and terpenoids. It is caused by the fact that this class of compounds is one of the majority BAS of the genus *Geranium* L. These materials are also resistant to oxidation and other changes (isomerization, cyclization, etc.), and are easily isolated from plants. Mass spectra of these compounds are present in the libraries databases for chromatography-mass spectrometry studies.

Table 1: Specific volatiles substances in grass of studied species of the genus *Geranium L.*

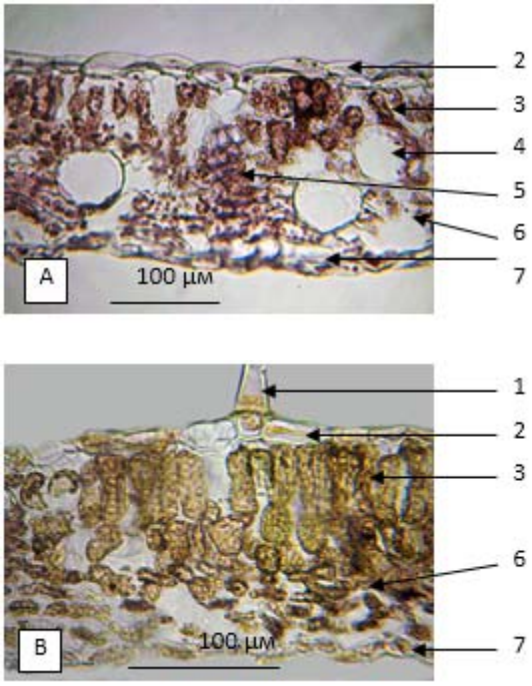
G. robertianum L.	G. sibiricum L.	G. sanguineum L.	G. macrorrhizum L.
Farnesylacetone	Sabinyl acetate	Phytol	γ- Elemene
Chrysanthenone	Germacrene D	2,3-Dehydro-α-ionone	β- Elemene
p-Cymen-8-ol	2-methoxy-4-vinylphenol	β- Ionone	Germacrene B
Verbenone	Tetracosane	β- Ionone -5, 6- epoxyde	
Isopiperitenone		trans-2-Hexenal	
Humulene epoxyde		trans-2- Heptenal	
1,8-Menthadien-4-ol		2,6-Nonadienal	
1,3-Cyclopentanedione		Nona-2,4,6- trienal	
Metylpenthadecane		6-Metyl-5-heptene-2-on	
		3, 5, 11, 15-Tetrametyl-1-hexadecene-3-ol	
Indole		Metyltetradecane	

The anatomical studies have shown that the leaves of considered species of the genus Geranium are amphistomatous, with dorsoventral mesophyll. They have stomata of anomocytic type. The stomata on the abaxial surface (for all species, except G. *sanguineum*) are larger by size and by quantity per 1mm^2, compared with adaxial surface. The epidermis cells have strongly flexuous shape. The projections of epidermal cells area are elongated. The tortuosity of epidermal cells is stronger, and the number cells on 1 mm^2 is much larger on the abaxial side than on the adaxial side of epidermis surface for all species except G. sanguineum (table 2). The upper and lower epidermis are single layer, covered with cuticle. In all studied species the thickness of upper epidermis is larger than the thickness of lower one. There are glandular and simple trichomes on lamina. The number of glandular trichomes on abaxial surface is higher relatively to adaxial surface. The palisade mesophyll consists of two layers of cells and takes a larger percentage of the leaf structure compared to the spongy mesophyll (fig. 1A-D). The latter contains small intercellular spaces. The vascular bundles are collateral type. The strengthening tissue is represented by sclerenchyma fibers of primary phloem and by collenchyme facing of vascular bundle on abaxial side of the leaf.

At the same time, it is worth to highlight some differences in the structure of leaves of the compared species. Thus, for G. macrorrhizum, the number of glandular trichomes (consisting of 2-celled stalk and capitulum) is the greatest on the upper surface of the leaf, compared with other species, and for G. robertianum – the respective number is the least (Table 2, fig. 2B). On the lower epidermis, this index is the highest for G. sanguineum and G. macrorrhizum and the lowest

for G. robertianum. The different representatives of the genus do not differ by the size of such trichomes. Also the specific distinction of species G. macrorrhizum and G. robertianum is a presence (on both surfaces of the leaf) of glandular trichomes with the length (in average) of 633.6 and 732.36 microns respectively, which consist of 5-celled stalk, placed at 6-7 celled basis, and capitulum (Fig. 2A). Besides the glandular trichomes, G. macrorrhizum has a great number of idioblast-cells – conceptacles of essential oils (Fig. 1 A). The latest are almost absent in other studied representatives.

The non-glandular trichomes in *G. macrorrhizum,* as in *G. robertianum*, found only in veins and are absent on the lamina between them. Furthermore, in *G. macrorrhizum* non-glandular trichomes are in veins on both sides of the lamina, and in *G. robertianum* only on abaxial side. Then in *G. sibiricum* and *G. sanguineum* the simple trichomes cover the entire lamina, in the latter they are much more than in *G. sibiricum*. Also there is a large variance of size of simple trichomes - from 76.8 to 664 mm in *G. sanguineum* (Fig. 2C).



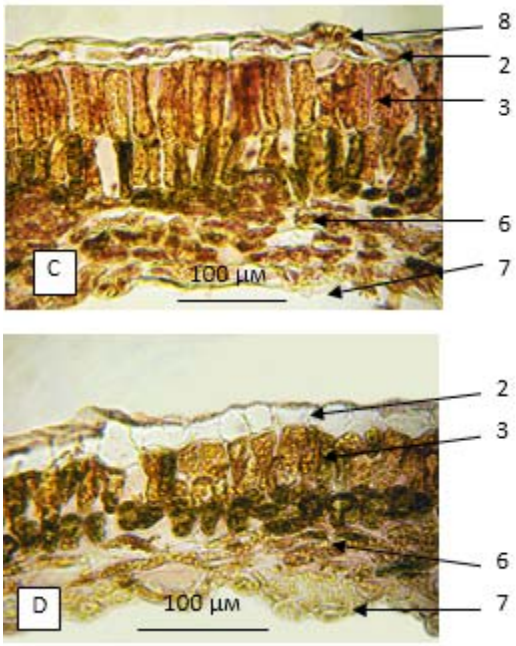


Fig 1: Cross section of lamina: A) *G. macrorrhizum,* B) *G. sibiricum,* C) *G. sanguineum,* D) *G. robertianum:* 1- simple trichome, 2- upper epidermis, 3 - palisade mesophyll, 4 – idioblast, glandular trichome, 5-vascular bundle, 6 - spongy mesophyll, 7- lower epidermis, 8- stoma.

The stomata index on the whole is the greatest in *G. robertianum*. The increasing of stomata index on abaxial surface in *G. sanguineum* is offset by the decreasing of this index on adaxial surface. Among the founded differences, comparing to other species, it is worth to mention significantly lower thickness of the upper and lower epidermis for *G. sibiricum*, the largest thickness of palisade parenchyma for *G. sanguineum* and the lowest thickness of palisade parenchyma for *G. sanguineum*. It would be interesting to mention that at the larger size of leaf lamina in *G. macrorrhizum* in comparison to

Table 2: Morphometric parameters of leaves of studied species of the genus Geranium L.

	G. macrorrhizum	*G. sibiricum*	*G. sanguineum*	*G. robertianum*
lamina				
Adaxial surface				
1	22,62±6,18	24,10±3,24	30,72±2,9*	23,68±2#
2	15,26±3,84	15,17±3,14	22,45±1,4*^	16,96±1,2#
3	46,86±15,1	43,43±14,7	37,67±14,8*^	32,67±8,7*^
4	798,86±98	758,86±112	1038,7±64*^	454,67±72*^#
5	0,055	0,054	0,035	0,067
6	29,71±12,3	18,57±7,2*	20,33±9,7*^	11±4*^#
abaxial surface				
1	25,89±2,89	24,35±2,5*	26,13±2,6^	26,08±1,9*^
2	19,26±3	17,02±1,8*	19,41±2^	19,36±1,6
3	176±36	288±32,8*	212,33±37*^	151,33±40*^#
4	1178,3±129	1427±157*	802,7±89,3*^	718,7±105*^#
5	0,129	0,168	0,209	0,174
6	30,86±11,3	23,43±4,7*	46±13*^	16,33±8,2*^#
7	20,72±6,6	17,98±3,4	20,33±3,2^	24,18±5,3^#
8	15,29±2,8	12,62±2,3*	14,58±2,5^	16,71±2,6^#
9	65,07±11,7	81,78±19*	106,84±13*^	82,13±15,4*#
10	48,71±5,1	46,04±12,4	65,42±13,3*^	40,89±11,3*#
11	154,49±9,9	157,81±23	198,93±21*^	162,31±25,4#
12	358,67±19	292,9±175*	313,35 ±218	376,37±68,5^
13	48±12,5	45,26±3,4	48,71±6,5	48,8±5,1
14	633,6±31,7	-	-	732,36±38,3
petiole				
15	44,42±7,2	54,04±9,8*	52,33±8,7*	60,6±4,9*^#
16	-	148,8±60,7	159,06±47	-
17	423,6±24,2	-	-	338±23,1
18	-	-	515±130,6	-
19	29,26±5,3	24±4*	21,28±1,9*^	48,16±7,6*^#
20	8	4	4	3-4
21	1-2	1	1	0-1

1-Length of stomata µm, 2 - Width of stomata, um, 3 - Amount of stomata pcs. / 1 mm^2 , 4 - No. of epidermal cells pcs. / 1 mm^2, 5 - stomata index, 6 - Amount of gland. tryhom, pcs. / 1 mm^2, 7. Thickness of upper epidermis, µm, 8 - Thickness of lower epidermis, µm, 9 - Thickness of palisade mesophyll, µm, 10 - Thickness of sponge mesophyll, µm, 11 - Thickness of leaf lamina, µm, 12, 16 - Length of simple unicellular trichom, µm, 13, 15 - Length gland. trichomes of 2-celled stalk, µm, 14, 17 – Length of gland. trichomes on pluricellular stalk, µm, 18 – Length of simple pluricellular trichomes, µm, 19 - Thickness of epidermis, µm, 20- Number of vascular bundle, pcs. , 21 - Number of layers collenchyme, pcs.

* - P<0.05 (compared with *G. macrorrhizum*); ^ - P <0.05 (compared with *G. sibiricum*);

- P <0.05 (compared with *G. sanguineum*).

G. sibiricum, the thickness of their leaf is similar. We suppose that such structural organization can be provided by increase of the thickness of the upper and lower epidermis, increase of the layers and the length of collenchyma and sclerenchyma vascular bundles facing in *G. macrorrhizum*. *G. sanguineum* has much thicker lamina compared to the other studied species (fig.1C).

The petiols of studied species have a similar structure: a single-layered epidermis with thick cuticle, 1-2 rows of lamellar collenchyme, parenchymal tissue, vascular bundles of collateral

type. The distinctive features in *G. macrorrhizum* and *G. robertianum* (besides glandular trichomes with 2-celled stalk) are the presence of glandular thrichomes with pluricellular stalk, and absence of non-glandular trichomes. The longest glandular trichomes with 2-celled stalk are discovered in *G.robertianum*, and shortest in *G. macrorrhizum* (Table 2). The characteristic feature of petioles of G. sanguineum is a presence of a single simple multicellular trichomes.

The simple unicellular trichomes are present only on petioles of *G. sibiricum* and *G. sanguineum*, the latter has a great number of them. The petioles of *G. macrorrhizum* are larger in diameter. This causes an increase in the number of layers of collenchyma, and the thickness of the epidermis, performing the mechanical function.

The number of vascular bundles in all investigated representatives of *G. macrorrhizum* twice as much compared to the other studied species. In *G. robertianum* are observed, sometimes, 3 and 4 vascular bundles, collenchyma is very poorly marked, indemnifying with the significantly thicker epidermis. The thickening of the epidermis is primarily caused by the thickness of the cell wall and cuticle.

The obtained results have a scientific and practical novelty. The specific components of volatile compounds can be a chemotaxonomic feature of the studied species within the same genus and be recommended as a matter - markers to identify and establish the identity of species of the genus Geranium L. in phenophase of mass flowering. The discovered differences also testify about a great similarity in anatomical features of the species *G. macrorrhizum* with *G. robertianum,* and *G. sanguineum* with *G. sibiricum*. The studied species most differ for the variety, size and density of trichomes arrangement, the number of vascular bundles in petioles.

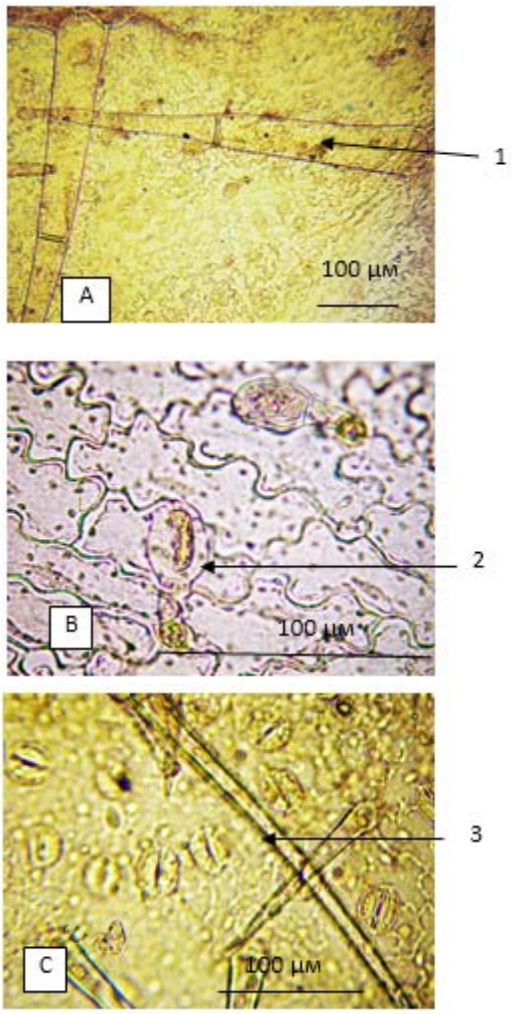


Fig 2: The epidermis of the leaf: A) glandular trichomes on multicellular stalk (1) *G. macrorrhizum,* B) short glandular trichomes**,** (2) *G. macrorrhizum*, C) unicellular non-glandular trichomes (3) *G. sanguineum.*

The individual distinct features are a large number of idioblast in the leaves and 8 vascular bundles in petioles of *G. macrorrhizum*, and the presence of non-glandular multicellular trichomes on the petioles of *G. sanguineum*. The revealed anatomical differences can be used for identification of species in the raw material. Based on anatomical data, the investigated species can be rank by decreased productivity of ether-oil substances as follows: *G. macrorrhizum, G. robertianum, G. sanguineum, G. sibiricum.* The installed ranking has a positive correlation with the date of quantitative content of amount of volatile compounds in grass of the studied species. The amount of volatile compounds in the

grass of *G. macrorrhizum* - 2866,6 mg / kg, *G. robertianum* - 1165, 7 mg / kg, *G. sanguineum* - 393,5 mg / kg, *G. sibiricum* - 161,1 mg / kg.work.

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