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### Research on Quantitative Content of Lectins in Plants of the *Geranium L. Genus*

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Results of a comparative study of the quantitative content of lectins in four plant species of the *Geranium L.* genus of flora of Ukraine. Quantitative lectin content was determined by the reaction of hemagglutination of human erythrocytes.

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**Keyword:** Species of Plants of the Genus *Geranium L.*, Lectins, Reaction, Hemagglutination.

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#### 1. Introduction

Plants of the genus *Geranium L.* are found throughout the world, especially in the temperate climate zone of the northern hemisphere. There are approximately 300 species of the genus *Geranium L.* in the world and more than 550 varieties of different species of the genus. In CIS countries, approximately 50 species grow. Flora of Ukraine includes 24 species of the genus *Geranium L.*<sup>[1,2]</sup>.

The biologically active substances (tannins, flavonoids, essential oils), and pharmacological activity of plant species of the genus *Geranium L.* are the subject of scientific research in various countries around the world<sup>[3-7]</sup>.

Information regarding the content of lectins in plants of the genus *Geranium L.* is almost completely lacking. The only mention of the study of lectins in the species of the genus

*Geranium L.* - *G. caroliniaum L.* is found in the article by J.T. Hardman (1983)<sup>[8]</sup>.

Lectins are a group of proteins of non-immune origin with the ability to reverse and selectively bind carbohydrates and carbohydrate markers of biopolymers, without changing their covalent structure<sup>[9,10]</sup>.

The first lectin was discovered by Dr. Peter Hermann Stillmark at the University of Dorpat (Tartu) University in 1888. He studied the toxicity of the seeds and oil from the seeds of *Ricinus communis L.* and found that they contained a toxic protein that causes, even in small quantities, agglutination of erythrocytes and their hemolysis. This protein was named ricin. Stillmark's work initiated worldwide research on lectins. Currently, many lectins have been discovered, including ricin, abrin, WGA (wheat germ agglutinine), canavaline (lectin from the

seeds of the horse bean - *Canavalia ensiformis* L. (DC)), etc. <sup>[9]</sup>.

The physiological activity of some lectins involves the use of plant extracts to regulate biochemical processes in the human body and to increase its immunity and capacity to fight cancerous tumors <sup>[12]</sup>. As a result, lectins are the subject of ever-greater interest on the part of pharmaceutical researchers. Therefore, the search for new lectins and study of their properties is still an urgent task for modern medicine and pharmacology.

## 2. The Aim of the Study:

The aim of our study was to determine the quantitative content of lectins in four plants of the genus *Geranium* L. of flora of Ukraine.

The objects of study were grasses and roots of four species of the genus *Geranium* L.: *Geranium robertianum* L., *Geranium sibiricum* L., *Geranium sanguineum* L. and *Geranium macrorrhizum* L. Raw material was collected in the early phase of vegetation and the phenophase of mass flowering on experimental plots in the Academic O.V. Fomin Botanical Garden (Kiev, Ukraine) in 2012.

## 3. Materials and Methods

The plant material used in the experiments was dried at room temperature and crushed to a particle size of  $\approx 1$  mm. The generally accepted method for the determination of lectins is the reaction of hemagglutination (RHA) erythrocytes. The analyses were carried out according to the guidelines of M.D. Lutsyk <sup>[11]</sup>.

A corresponding sample was homogenized with buffered saline (PBS) at a ratio of 1:5. PBS has

the following composition: 8 g NaCl, 0.2 g of KCl, 1.15 g of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  was dissolved in 1 liter of distilled water and pH was raised to 7.4 with the aid of 1 g of HCl or NaOH. The homogenate was filtered and the lectin content determined in the filtered extract by means of the hemagglutination reaction, using a 2% suspension of Group IV (AB) native human red blood cells under the ABO system. In order to determine lectins, we prepared a series of successive double dilutions of the extract on standard slides for immunological reactions. 5 ml of PBS is added to a series of U-shaped apertures then 0.05 ml of the experimental extract is added to the first aperture. 0.05 ml of the compound is transferred from the first hole to the second, 0.05 ml from the second hole to the third and so on. We obtain a series of dilutions of the extract at the second, fourth, eighth, sixteenth, etc. repetitions. 0.05 ml of a suspension of red blood cells is added to each hole and left at room temperature for 30-40 minutes. In the absence of agglutination, the red blood cells gathered in the center of the hole in clearly defined red spots. The agglutinated red blood covered the entire bottom of the hole in a uniform layer. The lectin titer matches its solution in the remaining holes, in which we observed agglutination.

## 4. Results and Discussion

The results of these studies provided in Table 1 show that the estimated values of the hemagglutinative activity of the study samples differ significantly among themselves.

**Table 1:** Hemagglutinative reaction values (RHA titer) of extracts of plant material

Sample	Organ studied	Estimated values (RHA titer)
<i>Geranium robertianum</i> L.	grass (early vegetation)	no agglutination observed in wells
	rhizomes (early vegetation)	2
	grass (flowering)	no agglutination observed in wells
	rhizomes (flowering)	2 <sup>1</sup>
<i>G. sanguineum</i> L.	grass (early vegetation)	2 <sup>2</sup>
	rhizomes (early vegetation)	2 <sup>7</sup>
	grass (flowering)	2 <sup>4</sup>
	rhizomes (flowering)	2 <sup>9</sup>

<i>G. macrorrhizum L.</i>	grass (early vegetation)	2 <sup>5</sup>
	rhizomes (early vegetation)	2 <sup>7</sup>
	grass (flowering)	2 <sup>7</sup>
	rhizomes (flowering)	2 <sup>9</sup>
<i>G. sibiricum L.</i>	grass (early vegetation)	2 <sup>2</sup>
	rhizomes (early vegetation)	2 <sup>5</sup>
	grass (flowering)	2 <sup>4</sup>
	rhizomes (flowering)	2 <sup>7</sup>

There was a significant difference in terms of lectin content in various organs and different species of the genus *Geranium*, particularly in the aerial parts of *G. sanguineum L.* (2<sup>2</sup>) RHA titer values are 7 positions lower than those of rhizomes (2<sup>9</sup>); lectin content values in the aerial parts of *G. macrorrhizum L.* (2<sup>5</sup>) less than 2 positions with those of rhizomes (2<sup>7</sup>), the corresponding proportions of the values observed in *G. sibiricum L.* In *G. robertianum L.* grass, lectins were absent and only found in small amounts in the rhizomes. It is interesting to note that lectin content in the studied plant material increases during seasonal growth (from the beginning of vegetation to plant flowering). This dependence is observed in all studied species of the genus *Geranium L.* It should be noted that the highest assay values of lectin content were observed in roots of the species *Geranium sanguineum L.* (RHA titer - 2<sup>9</sup>), *G. macrorrhizum L.* (RHA titer - 2<sup>9</sup>) and *G. sibiricum L.* (RHA titer - 2<sup>7</sup>).

It should be noted that we found lectin content in dried and crushed raw materials, which can save time and be used over a longer period, not only during the plants' growing season.

Thus, we studied lectin content in samples of plant material (*Geranium L.*) by comprehensive phytochemical studies of various groups of biologically active substances in raw plants of the genus *Geranium L.*

The results of the study must be taken into consideration in further studies concerning the possibilities of using the plants studied as sources of raw materials for pharmaceutical substances.

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