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Biotechnology

Distribution of Mannose-Specific Lectin in Physiologically Distinct Parts of the Mountain Plant Solomon's Seal (*Polygonatum obtusifolium* Miscz. ex Grossh.)

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ABSTRACT. Distribution of mannose-specific lectins has been studied in physiologically distinct underground and aerial parts of the Georgian endemic plant species - the mountain plant Solomon's seal (*Polygonatum obtusifolium* Miscz. ex Grossh.). The highest content of lectins was registered in the underground storage organ of the plant – the rhizome collected in autumn. Content of lectin in rhizome was 2 times higher than in the root and 10 times higher than in the bud. Content of lectins in all underground parts of the Polygonatum plant collected in spring is equal and it is 10 times lower than in the same organs, collected in the autumn period. Extracts of aerial parts of Polygonatum (stem, leaf, flower and seeds) made in spring caused lysis of rabbit erythrocytes, thus making impossible detection of hemagglutination activity in their extracts. Hemagglutination activity of Polygonatum lectins towards trypsin-treated rabbit erythrocytes was 8-10 times higher than towards native rabbit erythrocytes. The obtained results show that the manifestation of hemagglutination activity of Polygonatum lectin requires screening of mannose-specific receptors on rabbit erythrocyte surface by the method of treatment with trypsin. Data presented in this study unambiguously evidence that lectins from Polygonatum plant do not react with native erythrocytes of any of human blood groups I(0); II(A); III(B) and IV(AB) and, correspondingly, do not possess agglutination activity towards them. *© 2014 Bull. Georg. Natl. Acad. Sci.*

Key words: *Polygonatum obtusifolium* Miscz. ex Grossh.*, lectins, localization, distribution.*

Lectins belong to the versatile groups of polypeptides with the capacity of specific binding with saccharides. Lectins mainly are associated with a wide spectrum of pathological processes proceeding in an organism. A number of researches aimed at identification of new lectins and study of their biological properties is being intensively increasing [1].

Georgian endemic, medicinal plant *Polygonatum obtusifolium* - Solomon's seal - widely used in folk medicine in Georgia, with a broad spectrum of curative properties to different diseases, has been chosen as object of investigation.

Infusions of Solomon's seal are widely used for the treatment of lung diseases, diseases of the upper respiratory tract, acute bronchitis, hepatic diseases, gastric ulcer, arthritis, radciulitis, sciatica, diabetes

Table 1. Result of analysis of extracts of underground parts: root, rhizome and bud of mountain Solomon's seal (*Polygonatum obtusifolium*) obtained in spring: (C) – protein content, hemagglutination titre (T), lectin (hemagglutination activity) activity (HA), specific hemagglutionation activity – (SHA) and lectin content (LC)

Autumn					
Underground parts of Polygonatum	C mg/ml	T	HA mg/ml	SHA mg/ml	LC
Root	0.85	2^5	0.007	37.6	121.4
Rhizome	0.76	2^6	0.003	84.2	253.3
Bud	0.98	2^3	0.031	8.16	31.4
Spring					
Root	1.56	2^4	0.070	10.25	22.28
Rhizome	1.63	2^5	0.045	19.63	36.22
Bud	1.70	2^2	0.080	2.35	21.5

mellitus, gynaecological diseases, impotence, healing of wounds, abscesses, dermatitis and other diseases. Solomon's seal is especially intensively used for the treatment and prevention of tumours. Polygonatum is also used for improvement of weakened immune system, thus contributing to the rejuvenation of an organism and raising its potential in the senile age. Despite this active substances, causing curative effects actually remain unstudied so far and not incorporated in the modern medicinal practice [2].

The above reason prompted us to chose the Georgian endemic species of mountain Polygonatum (Solomon's seal) as object of study of biologically active lectins.

The present work implies the second necessary step in the research of lectins of the mountain Polygonatum. It is the study of distribution of mannose-specific lectins in physiologically sharply distinct underground and aerial parts of the plants and establishment of their properties.

Objects of Investigation and Methods.

Aerial parts (stems, leaves, flowers, seeds) and underground organs (roots, rhizomes, buds) were used as objects of investigation. Plant material was harvested in autumn, when the plant passes to the dormant state, and in spring, in the stage of active growth and development of the plant.

Separate underground and aerial organs of Polygonatum plant were crushed and homogenized in the homogenizer of the blender type or on the porcelain cup. Extraction for the obtaining of soluble protein fraction was made using the extracting solution: 0.9% NaCl, 0.1% b-mercaptoethanol (b-M), 0.04M K+ phosphate buffer, pH 7.4; ratio between the raw material and extracting solution (w/v=1/5). For the maximum extraction of soluble proteins the homogenate was placed to the magnetic stirrer for 30 minutes in conditions of room temperature (20-25ºC). The extract was filtered through the double gauze and the filtrate was centrifuged at 16000 r/m for 15 minutes. The supernatant was filtered through the Whatman CF/C and Sinpor-0.45-0.22mM filter. Excess inorganic ions were removed by means of dialysis on G-10 Sephadex column (50x2.7 cm). Extracts were kept in the fridge at +4ºC.

Lectin activity was determined visually on immunological plates using the hemmagglutination test on native and trypsin-treated rabbit erythrocytes and native erythrocytes of human blood of O, A, B and AB groups, by the microtitration method by Takatsy [3]. Lectin activity, hemagglutination activity (HA) was evaluated according to the minimum

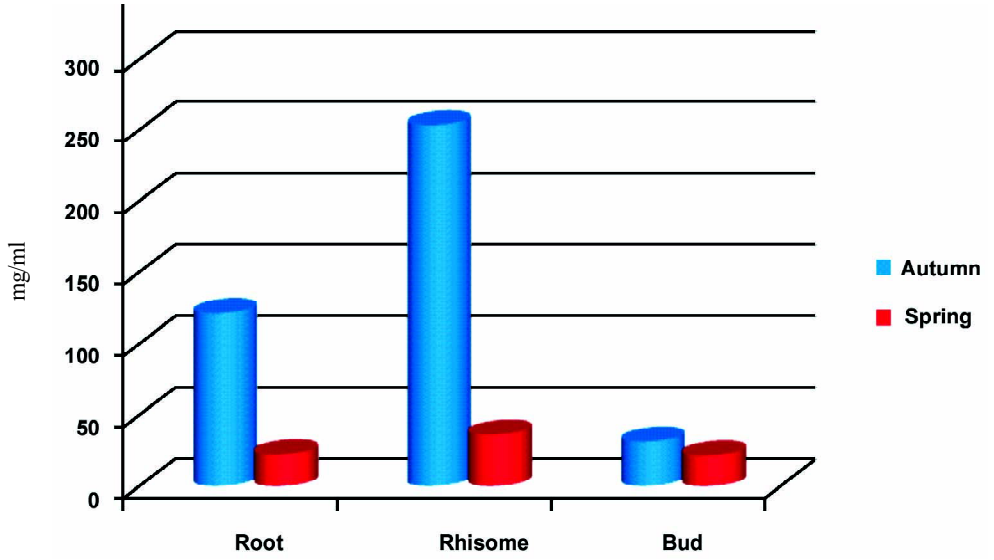

Fig. Content of lectins in underground parts of Solomon's seal (*Polygonatum obtusifolium*) in autumn and spring

concentration of protein (mg/ml), which caused agglutination of trypsin-treated rabbit erythrocytes (Fig.). According to the results of research also the hemagglutination titer (T) was determined. This index is indicative of the highest dilution of the agglutinin, which still causes visual aggltutination of erythrocytes. T=2n, where n is number of wells, where agglutination is visible. For evaluation of lectin activity also specific hemagglutination activity – (SHA) (mg/ml) was determined, which is a reverse value of the lectin activity. It represents the maximum dilution of 1 mg protein, which still causes agglutination: SHA=T/C, where C is protein concentration in mg/ml units. Lectin content (LC) was judged about by the ratio of total protein concentration to the lectin activity (conventional Hemagglutination Unit (HU). LC=C/HA.

Protein content was determined according to [4].

Results and Discussion

In the first series of experiments we investigated distribution of mannose-specific lectin (SABA-1) in physiologically distinct underground and aerial parts of mountainous Polygonatum (Solomon's seal) and studied its properties (Table 1).

As seen from the Table, protein content is the highest in the extract of autumn bud. At the same time the highest values of such lectin indexes as T, HA, SHA and LC were registered in root and rhizome extracts. In particular these values are 5 times higher in root extracts and 10 times higher in rhizome extracts compared with the bud extract.

Data presented in the Table show that in spring, in the period of active vegetation and growth, the protein content is almost 2 times higher in underground parts of Polygonatum plant, though lectin indexes T, HA, SHA and LC are 10 times less in root and rhizome extracts, and only 2 times reduced in the bud extract compared with autumn period.

In separate experiments the aerial parts: stem, leaf, flower and seeds of Polygonatum plant harvested in spring were studied. The study revealed that extracts form aerial parts of Solomon's seal plant caused lysis of rabbit erythrocytes. Due to this it was impossible to reveal hemagglutination activity in these extracts. As it is known from scientific literature, Solomon's seal plant contains big amount of saponins [5], which in our case caused active lysis of erythrocytes. Despite this, the method used by us did not yield evidence, proving absence of lectins in aerial parts of the plant. Further experiments require different approaches for getting precise answers.

Table 2. Lectin activity (C, T, HA, SHA) of extracts of underground parts of Solomon's seal (*Polygonatum obtusifolium*) , harvested in autimn, towards native and trypsin-treated rabbit erythrocytes

Underground parts of Polygonatum	Rabbit erythrocytes	T	HA	SHA	C
Root	Native erythrocytes, without treatment with trypsin	2^3	0.08	5.1	1.56
Rhizome		2^3	0.029	10.5	0.76
Bud		-	-	-	1.70
Root	Trypsin treated erythrocytes	2^5	0.007	37.6	0.85
Rhizome		2^6	0.003	84.2	0.76
Bud		2^3	0.031	8.16	0.98

The results of research allow to conclude that the highest content of lectins is registered in underground part - rhizome of Solomon's seal, collected in autumn. It should be noted that its physiological state corresponds to the transition to dormancy.

The Figure showing distribution of lectins in underground parts of Polygonatum plant harvested in autumn and spring, demonstrates the conclusion, drawn on the basis of results, presented in the Table 1.

As seen from the Figure lectins content is the highest in autumn rhizome which 10 times exceeds lectin content of spring rhizome. The obtained results point to the high mobility of Solomon's seal rhizome lectin. Positive correlation of the latter with physiological dormancy of the plant allows us to make supposition about protective biological functions of lectins in rhizome tissues of Polygonatum in autumn.

In the next series of experiments lectin indexes (C, T, HA, SHA) towards native and trypsin-treated rabbit erythrocytes were determined in extracts of underground parts of Polygonatum plant.

According to scientific data some lectins do not require treatment of erythrocytes with trypsin , isolated from the rabbit peripheral blood for manifestation of maximum hemagglutination activity [6]. These lectins equally express hemagglutination activity to both native and trypsin-treated erythrocytes. Because of this, study of lectin activity using native erythrocytes simplifies the method of determination of hemagglutination activity and allows to save time and chemicals. Considering this in the next series of experiments indexes of lectin activity (C, T, HA, SHA) towards native and trypsin-treated rabbit erythrocytes were studied in underground parts of Solomon's seal, harvested in autumn.

Table 3. Hemagglutination activity extracts of underground parts of Solomon's seal (*Polygonatum obtusifolium*) harvested in autumn towards native erythrocytes of human peripheral blood of I(0); II(A); III(B) and IV(AB) groups

Underground parts of Polygonatum	C mg/ml	Hemagglutination of native erythrocytes of human peripheral blood			
		Group I (0)	Group II (A)	Group III (B)	Group IV (AB)
Root	0.85	-	-	-	-
Rhizome	0.76	-	-	-	-
Bud	0.98	-	-	-	-

As seen from Table 2 all indexes (C, T, HA, SHA) of lectins extracted from underground parts of Solomon's seal plant, collected in autumn were 8-10 times higher in case of using trypsin-treated erythtocyes as compared with cases, where native erythrocytes were applied. The obtained results indicate that for the necessary condition for the manifestation of hemagglutination activity of Solomon's seal lectins is screening of mannose specific lectins on the surface of rabbit erythrocytes by means of treatment with trypsin.

Hemagglutination activity of autumn extracts of underground parts of Polygonatum towards native human erythrocytes was studied in special experiments.

Data presented in Table 3 unambiguously show that lectins isolated from Solomon's seal (*Polygonatum obtusifolium*) do not react with native erythrocytes of either group of human blood and, correspondingly, do not have the capacity for their agglutination. Literary data show that many plant lectins reveal immunotropic, anticancerogenic, antimicrobial and other interesting biological activities in experiments carried out in vitro, though their implementation in traditional medicine is limited due to toxic effects of lectins on human organism, among which is agglutination of erythrocytes in the blood.

The obtained results are significant in terms of future application of Solomon's seal (*Polygonatum obtusifolium*) lectins as curative means in medicine. Of special interest is the fact, established by us, that maximum content of Polygonatum lectin is registered in autumn period in rhizome, whose extracts, as it was mentioned in introduction, are widely used in folk medicine for the treatment of a wide range of diseases.

ბიოქიმია

მანოზა-სპეციფიკური ლექტინის განაწილება მცენარე მთის სვინტრის (Polygonatum obtusifolium Miscz. ex Grossh.) ფიზიოლოგიური მდგომარეობით მკვეთრად განსხვავებულ ნაწილებში

ნ. დუმბაძე, ნ. ალექსიძე, გ. ალექსიძე

საქართველოს საპატრიარქოს წმიდა ანდრია პირველწოდებულის სახელობის ქართული უნივერსიტეტი, თბილისი

საქართველოს ენდემურ მცენარე მთის სვინტრში (Polygonatum obtusifolium Miscz) შეწავლილ იქნა მანოზა - სპეციფიკური ლექტინის ზოგიერთი თვისება და მისი განაწილება ფიზიოლოგიური მგდომარეობით მკვეთრად განსხვავებულ მიწისქვეშა და მიწისზედა ნაწილებში. ნაჩვენებია ლექტინის ყველაზე მაღალი შემცველობა შემოდგომაზე მოპოვებული სვინტრის მიწისქვეშა ნაწილში - ფესურაში. ლექტინის შემცველობა ფესურაში 2-ჯერ უფრო მაღალია ვიდრე ფესვში და 10-ჯერ უფრო მაღალი ვიდრე კვირტში. გაზაფხულზე მოპოვებული სვინტრის მიწისქვეშა ყველა ნაწილებში

ლექტინის შემცველობა თანაბარია, თუმცა 10-ჯერ უფრო მცირეა შემოდგომაზე მოპოვებულ მიწისქვეშა ნაწილებთან შედარებით. გაზაფხულზე მოპოვებული სვინტრის მიწისზედა ნაწილების ღეროს, ფოთლის, ყვავილის და თესლების ექსტრაქტები ახდენენ ბოცვრის ერითროციტების ლიზისს, რის გამოც შეუძლებელი გახდა მათ ექსტრაქტებში ჰემაგლუტინაციური აქტივობის გამოვლენა ჩვენს მიერ გამოყენებული მეთოდით. სვინტრის ლექტინის ჰემაგლუტინაციური აქტივობა 8-10-ჯერ მეტი იყო ტრიპსინიზებული ერითროციტების გამოყენებისას, ვიდრე ნატიური ერითროციტების გამოყენების შემთხვევაში. მიღებული შედეგები მიუთითებენ, რომ სვინტრის ლექტინის ჰემაგლუტინაციური მახასიათებლების გამოსავლენად, აუცილებელ პირობას წარმოადგენს ბოცვრის ერიტროციტების ზედაპირის მანოზის შემცველი რეცეპტორების ეკრანირება ტრიპსინიზაციის მეთოდით. ნაშრომში წარმოდგენილია მონაცემები, რომლებიც ერთმნიშვნელოვნად მიუთითებენ, რომ სვინტრის ლექტინი არ რეაგირებს ადამიანის სისხლის არც ერთი **I(0); II(A); III(B)** და **IV(AB)** ჯგუფის ნატიურ ერითროციტებთან და შესაბამისად მას არ გააჩნია აგლუტინაციის უნარი.

REFERENCES

1. *A. Pusztai* (2008), Plant Lectins (Chemistry and Pharmacology of Natural Products). Cambridge University Press, 263.
2. *Khan, H., M. Saeed and N. Muhammad*. (2012), Pharmacological and phytochemical updates of Polygonatum. Phytopharmacology, 3: 286-308.
3. *Liener I.* (1976), Ann. Rev. Plant Physiol., 27: 291-319.
4. *Lowry O.H., Rosebrought N.J., Far A. L., Randall R.J.* (1951), J. Biol. Chem., 193 (1): 265-271.

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