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Original Research Article

A study of lectin activity in buds of Sophora japonica L

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Abstract

Purpose: *To identify lectins in Sophora japonica L. (green flower buds, fully formed flower buds, and flower buds as they begin to open) and to study their activity.*
Methods: *Lectin activity was studied using rat hemagglutination method. The protein concentration of the extracts of the agglutinate was determined using Bradford assay.*
Results: *Lectin activity of green flower buds was 1.61 ± 0.11 units/mg protein; fully formed flower buds, 1.81 ± 0.08 units/mg protein; flower buds as they began to open, and 2.05 ± 0.05 units/mg protein. The protein content of extracts from the buds of Sophora japonica L. collected at the stage of green flower buds, at the stage of formed buds, and at the stage of opening flower buds were 3.97 ± 0,04, 3.53 ± 0.07 and 3.13 ± 0.09 mg/ml respectively.*
Conclusion: *This study shows the existence of lectins in Sophora japonica L. buds studied at three different stages of development. The highest lectin activity and protein content are found in the stage of green flower buds.*

Keywords: *Lectins, Sophora japonica L, Flower buds, Ratuserytroagglutination*

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INTRODUCTION

The word lectin is derived from the Latin word legere, which means “to select.” Lectins are proteins that bind to carbohydrates and sugar-containing substances in specific and reversible ways, or that precipitate glycoconjugates [1-3].

In 1888, Stillmark found that extracts of castor beans seeds contained a toxic substance, ricin, that agglutinated human and some animal red blood cells. However, the modern age of lectinology started nearly 100 years later [4,5].

The lectins are divided into two major groups: endolectins (the body's own lectins) and exolectins (those that enter an organism from outside). The functions of lectins in nature have wide variety and are not fully understood, but all are based on their ability to recognize and bind carbohydrate parts of glycoconjugates in solution as well as in cells [6]. Lectins were initially found and described in plants of the legume family such as soybeans, beans, lentils, peas, peanuts, and other. These plants are main components of human nutrition in many countries, but in subsequent years more lectins have been isolated from microorganisms and also from animals [7]. For a long time the lectin-induced agglutination of cells has served as the most common assay to detect and quantify lectin activity in a variety of organisms [8-10].
Among the best characterized lectins that are found in legumes (but not the seeds) are those in

the bark and young shooting tissues of *Sophora japonica* L. These lectins belong to a very common natural group of carbohydrate-binding proteins. Lectins can selectively and reversibly bind to carbohydrates and to carbohydrate-containing biopolymers without breaking their chemical structures [11], and can specifically recognize of cytomembrane receptors in living organisms.

Previous studies have determined that galactose-specific lectin in the bark of *Sophora japonica* L., varies in content throughout the year and with the age of the tissue. During an average summer it measures 50 % less than in the winter. Higher accumulation of lectins in the tissues of young shoots has been observed in autumn and winter [12-14]. Also lectins were found in the fruits of *Sophora japonica* L. [15].

Consequently, establishing the presence of lectins in buds of *Sophora japonica* L. and determining their agglutinating activity constitute the main objective of this research.

EXPERIMENTAL

Plant material

Buds of *Sophora japonica* L. (Fabaceae family) in different stages (green flower buds, fully formed flower buds, flower buds as they begin to open) were collected in the Bakhchisarai area, Autonomous Republic of Crimea, from May to September during 2014 and 2015. Green flower buds are 5 mm long; rudimentary green petals are almost imperceptible; fully formed buds are 7 mm long, with well-marked, tightly serried light-green petals, which take ½ of the whole bud; flower buds as they begin to open are 9-10 mm long and have light-yellow petals visually separated from each other. Plant material was identified by Prof. Dr. Minarchenko V. M. (M. G. Kholodny Institute of Botany, Kyiv, Ukraine). The authenticated voucher specimens were kept in Herbarium of M. G. Kholodny Institute of Botany (Kyiv, Ukraine) with voucher specimen nos. 072290 KW, 119855 KW, and 119856 KW.

The temperature at harvesting time ranged between 30 and 35 °C. The raw material was dried in a well-ventilated dark place. Dried material samples were kept in a dry and dark place in multilayer paper bags at room temperature.

The study was conducted at the research laboratory, "Physiological Bases of Plant Productivity" NSC "Institute of Biology" of Taras Shevchenko National University in Kyiv, Ukraine.

Preparation of lectin-containing extract (LCE)

Lectin-containing extract (LCE) was prepared by weighing a 1 g sample of plant material which was then transferred into a porcelain mortar. 10 mL of physiological sodium chloride solution (0.9 %) was added and the plant material was ground for 5 min to attain a homogeneous state; this was filtered through a double-layer gauze filter and centrifuged at 3000 rpm for 15 min. Lectin activity (LA) was then tested using the supernatant that contained the lectin extract.

Preparation of erythrocyte suspension

Red blood cells (RBC's) were obtained from mature albino (non-linear) rats of both sexes, which were maintained in a controlled environment under standard conditions of temperature and humidity with an alternating light and dark cycle. The animals were fed with a standard diet, and water was provided *ad libitum*. All animals were in plastic cages with bedding, as required [16-18]. For experimentation animals were selected without external signs of diseases. The protocols were used in the present study were approved by the Ethic Committee at the O.O. Bogomolets National Medical University (Kiev, Ukraine) (approval ref. no 61/1.03.2014).

The experiment was performed according to the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, France, 1986) and according to "General Ethical Principles of Experiments on Animals" which was adopted the 1st National Congress on Bioethics (Kyiv, Ukraine, 2001) [17,18].

Blood was collected from the retro-orbital plexus without the use of an anticoagulant. RBC's were obtained from citrate solution of rat blood (one part blood to nine parts chilled 3.8 % solution of trisodium citrate). The mixture was centrifuged at 1500 rpm for 5 min. Citrate supernatant was removed, and this step was repeated four times under the same conditions. This washing process removes plasma proteins which would otherwise inhibit cell-lectin interactions. After the fourth washing procedure the cells were re-suspended in 25 mL of 0.15 M NaCl solution to make a suspension. The stable cell suspension was refrigerated at 4 °C until used.

Hemagglutination

The commonly accepted method for the determination of LA is the hemagglutination of erythrocytes and the presence of lectin in raw materials is revealed by agglutinative activity. LA was determined in raw materials by biological testing using the method of Pogorila *et al* [19] for hemagglutination. In brief, this method is based on the principle of agglutination mediated by lectins, namely the formation of aggregates throughnon-covalent inverse bonds of the active site of the protein subunits of the lectin with complementary receptors on rat erythrocyte membranes.

A 0.1 mL erythrocyte suspension of packed RBC's was added to a vial dispenser with 5 mL of saline solution and shaken gently. The reaction of hemagglutination (RHG) was performed on an immunological plate with U - shaped apertures. In each of the 8 holes of the vertical row, 0.05 mL of buffered saline solution was added (consisting of 1 L of water, 8 g sodium chloride, 0.2 g potassium chloride and 1.0 g of disodium phosphate). The solution was adjusted to pH 7.4 using 1 N HCl solution.

In order to avoid unacceptable artifacts related to inadequate removal of citrate blood plasma and to hemolysis of RBC's, we make a control to find the spontaneous deposit of washed RBC's. For this control we do not inject LCE into the test-system; instead we double the amount of saline solution to 0.1 mL with 0.05 mL of the suspension of rat erythrocytes (into a single vertical row of holes – A, in Figure 1). To prepare a series of successive two-fold dilutions of LCE, 0.05 mL of LCE was added in the first hole of the above-mentioned vertical row, stirred, and 0.05 mL was collected, which was then transferred to the next hole, number 2, stirred and 0.05 mL was collected, and then transferred to the following hole and so on, until we reached hole number 8, where 0.05 mL was also removed and discarded. Then, 0.05 mL of erythrocyte suspension was added to each hole and then the test system was left standing for 60 – 90 min at 25 °C. This testing of lectin substances from the plant extract was carried out three times.

Evaluation of hemagglutination

The intensity of the reaction of agglutination of erythrocytes (IRAE) was determined by the titre value of the individual holes. The absence of agglutination is indicated by a clear spot in the center of the bottom of the hole. The presence of agglutination is indicated when erythrocytes line the entire bottom of the hole ("umbrella") (Figure 1).

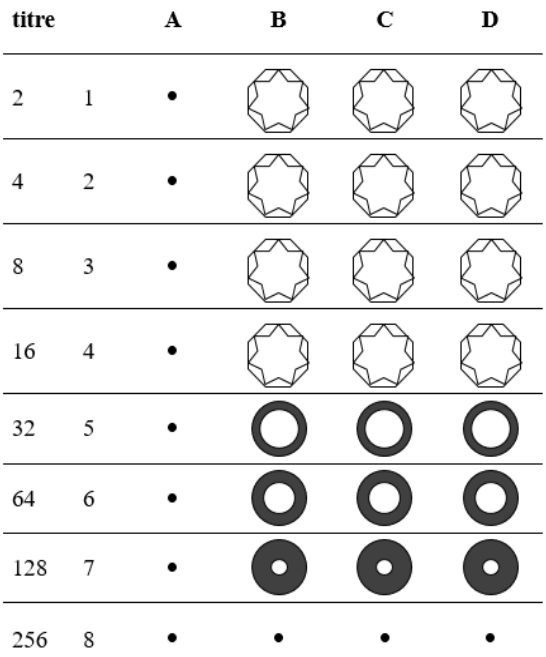

Figure 1: Evaluation scheme for titre. A – control; B - green flower buds; C - fully formed flower buds; D - flower buds as they begin to open

The hemagglutination titre value was determined as the maximum dilution of LCE at which erythrocytes were completely agglutinated: the higher the titre value, the higher the intensity of the reaction of agglutination of erythrocytes (Figure 1) [19]. LA was expressed as titre value over mg of protein.

Determination of protein content

To ascertain the protein nature of the agglutinating substance, the extracts were tested for the presence of protein [20,21]. The protein contents of the crude extract samples were determined using Bradford assay [20].

This method is based on the reaction of the dye Coomassie (Coomassie Brilliant Blue G-250) with protein to form a complex blue colour.

Determination of lectin activity

The unit of LA is the minimum amount of lectin that produces agglutination of RBC's. The calculations were performed as in Eq 1 [22].

$$LA = (T/C) \times V \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where LA = the unit of lectin activity (units/mg protein);T = titre value; C = protein concentration (mg/mL);V = sample volume (0.05 mL).

Table 1: Protein contents and lectin activity of raw materials of *Sophora japonica L.* in different bud stages

Bud stage	Titre[a]	Protein content (mg/ml)	LA (units/mg protein)
Green flower buds	128	3.97±0.04*	1.61±0.11
Formed flower buds	128	3.53±0.07*	1.81±0.08*
Opening flower buds	128	3.13±0.09*	2.05±0.05*

[a]*Titre is the lowest dilution that was positive for lectin activity; * = significant difference*

Statistical analysis

The results are expressed as mean ± standard deviation (SD, n = 3). The reliability was assessed by Student's test at $p < 0.05$. All statistical analyses were performed using one-way ANOVA with Prism 5 software (GraphPad USA). Differences were considered statictically significant at $p < 0.05$.

RESULTS

The results obtained in the analysis, summarized in Table 1, show that the estimated values of the hemagglutinative activity of the *Sophora japonica* L. buds differed significantly in their different stages.

The titre for all the studied extracts of *Sophora japonica* L. buds collected in different stages was 128. The protein content in extracts from buds of *Sophora japonica* L. collected at the stage of green flower buds was 3.97 ± 0.04 mg/ml ($p < 0.05$), in the stage of formed buds it was 3.53 ± 0.07 mg/ml, and at the stage of opening flower buds it was 3.13 ± 0.09 mg/ml ($p < 0.05$). Additionally, the LA of the buds of *Sophora japonica* L. at the stage of green flower buds, at the stage of formed buds, and at the stage of opening flower buds was 1.61 ± 0.11 units/mg protein, 1.81 ± 0.08 units/mg protein ($p < 0.05$), and 2.05 ± 0.05 units/mg protein ($p < 0.05$), respectively.

DISCUSSION

We determined the presence of lectins in *Sophora japonica* L. buds collected in different stages. The greatest activity of lectins was in buds collected in the green flower stage (1.61 ± 0.11 units/mg protein), as agglutination occurred with a minimum concentration of lectins.

This finding can be associated with physiological needs of plants, as lectins are directly involved in the growth of the plant; they help plants to form a protective response [1-3,22], which is very necessary during the formation of buds.

In addition, lectins are important for humans. They perform a wide variety of physiological functions by regulating cell adhesion during glycoprotein synthesis and thus control protein levels in blood; they influence the processes of lymphocyte activation in immune response [3,4]. Pure lectins are important in clinical trials; for example, they are used to determine blood types. We also note that one activity of lectins is the antitumor effect [23,24]. For future phytochemical and pharmacological studies of lectins from *Sophora japonica* L., buds should be collected exactly in the green flower stage, because this is the stage of maximum lectin activity.

CONCLUSION

The findings of this study proved the existence of lectins in *Sophora japonica* L. buds studied at various stages of development. The highest protein content was found at the stage of green flower buds. Additionally, LA was studied for *Sophora japonica* L. buds. The highest LA was also in the stage of green flower buds: 1.61 ± 0.11 units/mg protein.

DECLARATIONS

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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