ФІЗІОЛОГІЯ РОСЛИН

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M.V. Semenko, S.V. Pospelov, V.V. Onipko
Poltava National Pedagogical University named after V.G. Korolenko
2 Ostrogradskogo St., 36000, Poltava, Ukraine
Poltava State Agrarian Academy
1/3 Skvorody St., 36003, Poltava, Ukraine
max_semenko@ukr.net
ORCID 0000-0002-9168-4238
ORCID 0000-0002-2260-971X

ACTIVITY OF LECTINS OF ST. JOHN'S WORT (HYPERICUM PERFORATUM L.) IN THE ONTOGENESIS

The purpose of the proposed article is to evaluate the St. John's Wort as a raw material base of phytolectins; to study the content of proteinaceous compounds in different parts and organs, the terms of procurement of raw materials with a maximum accumulation of compounds.

The author of the article used the aboveground part of St. John's wort (Hypericum perforatum L.) of Topaz variety of the generative period of ontogenesis grown on experimental sites of the botanical garden of the Poltava National Pedagogical University named after V.G. Korolenko in 2016-2018 years. The material for the study was collected in the stages of sprout formation, budding, flowering and fruiting.

To accomplish the tasks, a method of evaluation of lectin activity by setting the hemagglutination reaction in immunological plates was used. The assessment was carried out visually on a five-point scale.

The article presents the results of the research of hematagglutinating activity of lectins of St. John's wort (Hypericum perforatum L.) in the ontogenesis. It was established that their maximum activity was determined during the period of budding and flowering. High activity was observed in leaf lectins throughout the vegetation period (11.8 – 19.7 points). In the stems, the level of agglutinins increased from the period of sprout formation (5.5 points) to flowering (16.3 points). In generative organs, the activity of lectins was maximal (19.7 – 23.3 points).

It is concluded that the above-ground part, collected during the period of full flowering, is a source of agglutinins of high biological activity.

Key words: *St. John's wort, lectins, hemagglutinating activity, ontogenesis, Hypericum perforatum L.*

Introduction. St. John's wort (*Hypericum perforatum* L.) is one of the leading medicinal plants in the pharmaceutical industry (Solohub, 2011). This, first of all, is due to its chemical composition and the fact that many medicines that are used to treat the gastrointestinal tract, liver (Mahmoud, 2018), kidneys, respiratory tract (Valvassori, 2018) are produced from the raw material of St. John's Wort. It is a part of many antibacterial, astringent, antiseptic remedies (Marrelli, 2016). The unique herbal properties of the St. John's wort are due to the complex action of phenolic compounds present in it, but this question has not been fully studied yet. In this regard, noteworthy are lectins – biologically active compounds of protein nature, capable of specific and reverse binding to carbohydrates and carbohydrate-containing compounds (Mamenko, 2014). Various properties of lectins promote their widespread use in biochemistry, histochemistry and in the creation of medicinal preparations (Pavlovskaia, 2017; Shakyrova, 2007).

At the same time, the evaluation of the St. John's wort as a raw material base of phytolectins was not sufficiently carried out. The content of proteinaceous compounds in different parts and

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organs, the terms of procurement of raw materials with a maximum accumulation of compounds need to be studied further. If many plants have a sufficient level of scientific research on these aspects, then for the St. John's wort they need detailed processing.

That is precisely why the relevance of the work is determined and the scope of our research is outlined.

Materials and methods. As a plant raw material, the aboveground part of St. John's wort (*Hypericum perforatum* L.) of Topaz variety of the generative period of ontogenesis grown on experimental sites of the botanical garden of the Poltava National Pedagogical University named after V.G. Korolenko in 2016-2018 years was used. The collection was carried out in the stages of sprout formation, budding, flowering and fruiting.

Laboratory research was conducted on the basis of the Department of Agriculture and Agrochemistry of the Poltava State Agrarian Academy.

The aerated dry raw material was ground, sieved on sieves with a diameter of holes of 1 mm and used for further extraction of lectins. To do this, one part of the raw material was poured into ten parts of the physiological solution (0.9% NaCl), infused for 2 hours at room temperature and filtered.

Evaluation of lectin activity was carried out by setting the hemagglutination reaction in immunological plates (Lutsyk, 1981). For this, 0.05 ml of physiological solution were added to each well of the plate, then 0.05 ml of extract was added and the series of successive two-fold dilutions was prepared. After that, 0.05 ml of a 2% suspension of washed red blood cells was added to each well and the plate was left at 25 ° C for 2 hours. The evaluation was carried out visually on a five-point scale (Holynskaia, 1982):

3 points – sharply expressed agglutination. Erythrocytes in the form of a thin film more or less evenly distributed at the bottom of the well;

2 points – moderate agglutination. Erythrocytes diverge on the bottom of the well at a distance of more than 2 mm in diameter, forming a ring with sharply expressed granularity at the edges;

1 point – weak agglutination. Erythrocytes diverge on the bottom of the hole at a distance of less than 2 mm, forming a ringl or disk;

0,5 points – minimal agglutination. A small clearance appears in the center of the aggregate of erythrocytes, which have settled on the bottom of the well;

0 points – no agglutination. Erythrocytes accumulate in the center of the well.

After a visual assessment of the agglutination in each well of the dilution series, the sum was counted in all wells where the reaction was determined. Thus, the maximum activity in eight wells can be: $8 \times 3.0 = 24$ points (Pospelov, 2012).

Results and its discussion. Analysis of the available literature showed that, despite the fact that the presence of lectins in St. John's wort is determined, many aspects remain poorly studied. The lack of systematic data on the dynamics of accumulation of lectins at different stages of ontogenesis of St. John's wort in conditions of Ukraine prompted us to research this issue. The article presents data on the evaluation of the activity of lectins in extracts of different parts and organs of St. John's wort of Topaz variety.

There is a general trend of high level of hemagglutinating activity of extracts of buds and inflorescences, as well as leaves. The lower numbers were characteristic of stems and fruits. At the end of the vegetation there is a gradual decrease in the activity of phytolectins.

Figure 1 shows the change in the activity of lectins in the leaves of St. John's wort. In the period of sprout formation, the agglutinating activity of leaf extracts amounted to an average of 19.7 points. During the budding, it dropped to 11.8 points, and further numbers rose again to 18.0 – 19.3 points. In our opinion, this indicates that during the sprout formation lectins are actively synthesized in the leaves, and during the budding period they are transported to the generative parts of the plant. Further (the period of flowering and fruiting), their number increases and remains relatively stable until the end of the vegetation.

The agglutinating activity of extracts of stems at the beginning of the vegetation was minimal (5.5 – 6.5 points), but with the development of plants increased and at the end of the vegetation reached its maximum (14.8 – 16.3 points). This fact suggests that lectins have an important transport function due to their ability to reversibly bind to oligo- and polysaccharides, which are also found in St. John's wort [6]. The benefit of this is evidenced by the fact that in the dry stems, the activity was found to be the same as during vegetation.

Generative organs were found to accumulate a large amount of lectins. Their activity was highest in the forming buds, and was 23.3 – 24.0 points (Fig. 2). During flowering, it decreased (21.8 points), and during the formation of fruits – up to 19.7 points.

It should be noted that the agglutinating activity of fruit extracts was high and amounted to 12.8 points.

Based on the data obtained, it can be assumed that leaves should be considered as the main place of synthesis, and then localization, of lectins in St. John's wort. With the formation and growth of the shoots, phytolectins can be transported to stems and buds. It is possible that a significant role in this is played by polysaccharides of St. John's wort, which facilitate both the effective transport of proteins, and the accumulation of them in different parts and organs.

Conclusions. As a result of the research, certain patterns of changes in the activity of lectins in the ontogenesis of St. John's wort of Topaz variety have been established. In plants of the second year of vegetation, starting with the flowering phase, high activity of lectins is characteristic for leaves (18,0 – 19,3 points) and stems (14,8 – 16,3 points). The maximal hemagglutinating activity is established in inflorescences extracts, especially during the budding period (23.3 points). Capsules of St. John's wort have an average of 12.8 points.

The above-ground part of the St. John's wort of Topaz variety, collected during the period of mass flowering, contains a significant amount of lectins and can be a raw material source of these unique protein compounds.

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М.В. Семенко, С.В.Поспєлов, В.В. Оніпко
Полтавський національний педагогічний університет імені В.Г. Короленка
Полтавська державна аграрна академія

АКТИВНІСТЬ ЛЕКТИНІВ ЗВІРОБОЮ ЗВИЧАЙНОГО (HYPERICUM PERFORATUM L.) В ОНТОГЕНЕЗІ

Метою пропонованої статті є оцінка звіробою як сировинної бази фітолектинів, вивчення вмісту білкових сполук у різних частинах і органах, терміни заготівлі сировини з максимальним накопиченням сполук.Автором статті було використано надземну частину звіробою звичайного Hypericum perforatum L. сорту Топаз генеративного періоду онтогенезу, вирощеного на дослідних ділянках ботанічного саду Полтавського національного педагогічного університету імені В. Г. Короленка в 2016-2018 роках. Матеріал для дослідження було зібрано у фази пагоноутворення, бутонізації, квітування і плодоношення.Для реалізації поставлених завдань було застосовано методику оцінки активності лектинів шляхом постановки реакції гемаглютинації в імунологічних планшетах. Оцінку проводили візуально за п'ятибальною шкалою.Автором статті описано результати досліджень гемаглютинуючої активності лектинів звіробою звичайного (Hypericum perforatum L.) в онтогенезі. Було встановлено, що їх максимальна активність визначалась у період бутонізації та квітування. Високою активністю характеризувалися лектини листків протягом усього вегетаційного періоду (11,8 – 19,7 балів). У стеблах рівень аглютинінів зростав від періоду пагоноутворення (5,5 балів) до квітування (16,3 бали). В генеративних органах активність лектинів була максимальною (19,7 – 23,3 бали).1У статті стверджується що надземна частина звіробою звичайного сорту Топаз, зібрана у період масового цвітіння, містить значну кількість лектинів і може бути сировинним джерелом цих унікальних білкових сполук.

Ключові слова: *звіробій звичайний, лектини, гемаглютинуюча активність, онтогенез, Hypericum perforatum L.*