

Researching of secondary metabolites of *Adonis vernalis*

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*The *Adonis vernalis* callus biomass was obtained in vitro using biotechnological method. Secondary metabolites were found in biomass extracts. The results of thin-layer chromatography and spectrometric analysis of *Adonis vernalis* extracts are indicated. Obtaining of secondary metabolites is promising for the pharmaceutical and cosmetic industry.*

Keywords: secondary metabolites, alternative methods of obtaining, callus biomass, *Adonis vernalis*, cultivation.

Introduction

Currently, it is relevant to search for new biologically active compounds and alternative methods for their obtaining. Valuable sources are vegetable raw materials and products of its processing. The value is determined by the content of both primary and secondary metabolites, in particular alkaloids, glycosides, tannins, saponins, phenolic compounds, vitamins, etc. Most of these substances are difficult to synthesize because they have a complex chemical structure, so the only place of location of biologically active substances is higher plants. The high consumer effect of drugs containing compounds of natural origin is caused by many advantages as low toxicity, high efficacy, low side effects, and the presence of secondary metabolites. The raw material base for obtaining these compounds is the natural resources of medicinal plants. Currently, the arsenal of medicinal plant material is limited, because anthropogenic influences are rapidly increasing, climatic conditions are changing and these greatly affect the biological features of the plants. Modern biotechnology methods offer solutions to the problem by obtaining biomass in vitro, as an alternative source of secondary metabolites. This method has many advantages: biomass grows under controlled conditions, is environmentally friendly, the method does not depend on climatic conditions, so biomass can accrue faster and year-round.

The medicinal plant *Adonis vernalis* has a special value as a source of secondary metabolites. This plant is threatened with extinction, so the use of the biotechnological method for the obtaining of callus biomass is appropriate and promising.

Adonis vernalis has high cardiological activity due to its content of cardiac glycosides (cymarin, K-strophanthin - β), coumarins. It also contains saponins, benzoquinones, flavonoids, organic acids, steroidal saponins, ascorbic acid, provitamin A, micro and macro elements.

The aim of the study

Determination of the qualitative and quantitative content of secondary metabolites in the obtained *Adonis vernalis* callus mass.

Materials and methods of research

The seeds of *Adonis vernalis* were used as raw materials to obtain explants in vitro. Seeds selected after flowering from natural germination location (Lviv region, Ukraine). Seeds were pre-stratified and sterilized with various agents (hydrogen peroxide (96%), sodium hypochloride (3%)) for further cultivation. The Murashige and Skoog (MS) medium was used for the introduction into the culture of the seeds without phytohormones.

The Murashige and Skoog medium was used to cultivate *Adonis vernalis*. This medium contains the necessary components for cultivation of explants [1]. Determination of callus biomass was carried out using a weight method. Identification and quantitative determination of the content of secondary metabolites in the obtained callus biomass was carried out in accordance with the methods used for plant material in accordance with the requirements of the State Pharmacopoeia of Ukraine.

At the first stage the content of cardiac glycosides in callus biomass was determined using specific reactions, thin-layer chromatography, commonly used methods of researching [2]. Thin-layer chromatography (TLC) was carried out on plates of the Scornful brand, 10 × 10 cm in size. Ethyl acetate-methanol was selected as the solvent system - water in ratio of 75: 10: 7.5.

Content of the sum of polyphenols, tannins, flavonoids was determined spectrophotometrically in terms of pyrogallol, rutin, pyrogallol [3].

Results

As a result of the research were selected the optimal conditions for induction of Calusogenesis *Adonis vernalis*, namely, the medium of MS with the corresponding phytohormones (IOC, NOC, kinetin), illumination 4000lk at a 16 hour photoperiod at a temperature of 22-25 °C and optimal cultivation time is 40 days. It has been established that the maximum biomass increase is 3.04 grams of dry matter per 100 ml of nutrient medium at 40 days of cultivation. The obtaining high-quality callus biomass was used for further research.

Qualitative analysis of the content of biologically active compounds in the callus biomass identified the presence of glycosides, flavonoids, tannins and polyphenols.

As a result of conducting TLC was identified cardiac glycosides (cymarin, strophanthin - β); phenolic compounds (gallic acid, myrethecin, (+) epicatechin, 3,4-hydroxybenzoic acid).

The quantitative content of the amount of polyphenols in the *Adonis vernalis* callus biomass is 2.31%, the amount of flavonoids is 2.04%, tannins - 0.83%.

Conclusions

The callus biomass of *Adonis vernalis* was obtained and its ability to prolong the growth and accumulation of secondary metabolites as cardiac glycosides, tannins, flavonoids and polyphenols was established.

References

- [1] A. Krvavych, R. Konechna, R. Petrina, M. Kyrka, N. Zayarnuk, R. Gulko, N. Stadnytska, and V. Novikov, "Phytochemical research of plant extracts and use *in vitro* culture in order to preserve rare wild species *Gladiolus imbricatus*", *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, vol. 5, no. 1, pp. 240-246, January - February, 2014. [Online]. Available: [https://www.rjpbcs.com/pdf/2014_5\(1\)/\[28\].pdf](https://www.rjpbcs.com/pdf/2014_5(1)/[28].pdf)
- [2] Derzhavna farmakopeya Ukrayiny. Derzhavne pidpryyemstvo "Naukovo-ekspertnyj farmakopejnyj centr". – I-e vyd. – X.: RIREG, 2001. – Dop. 1. – 2004. – 520 s.
- [3] Krvavych A.S., Konechna R.T., Novikov V.P., "Vyvchennya biologichno aktyvnyx rehovyn kosarykiv cherepytchastyx (*Gladiolus imbricatus*)", *Visnyk Nacional'nogo universytetu "L'vivska politexnika"*, vol. 787, S.217-220, 2014.