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EXPERIMENTAL PAPER

Obtaining and research of callus biomass of some plants of the family *Ranunculacae*

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Summary

Introduction: Research among the Ukrainian (Carpatian) flora's representatives is important for searching for new active compounds.

Objective: The study aims to determine the potential of callus biomass of *Delphinium elatum, Anemone nemorosa,* and *Pulsatilla alba* to become an analog of medicinal plant raw material for the use in the pharmaceutical industry, to investigate the composition, antioxidant activity and antimicrobial activity of extracts of the callus biomass of the above-mentioned plants.

Methods: The individual members of the family *Ranunculaceae (Delphinium elatum* L., *Anemone nemorosa* L., *Pulsatilla alba* Rchb.*)* were introduced into *in vitro* culture, and their callus induction was studied using the Folin-Ciocalteu spectrophotometric method, colorimetric analysis, antioxidant, and antimicrobial activity methods.

Results: The maximum content of flavonoids and phenolic compounds was observed in 40% hydro-ethanolic extracts of callus biomass and plant raw materials of *D. elatum*. 90.0% and 70.0% *D. elatum* extracts showed significant activity against Gram-positive microorganisms. 90.0/70.0/40.0% extracts showed significant activity against *Bacillus licheniformis.* 70.0 % extract showed significant antifungal activity against clinical and reference strains of *Candida albicans.*

Conclusion: Summarizing experimental results, it was proved that the callus biomass of *D. elatum, A. nemorosa, and P. alba* have potential as analogs of medicinal plant raw materials, both for the content of biologically active substances and biological activities.

Keywords: ***Ranunculaceae, callus, extracts, flavonoids and phenolic compounds, antimicrobial activity, antioxidant activity***

Słowa kluczowe: ***Ranunculaceae, kalus, wyciągi, flawonoidy i składniki fenolowe, aktywność antybakteryjna, aktywność antyoksydacyjna***

INTRODUCTION

The problem of biodiversity has been on the rise worldwide in recent decades due to violations in all components of the environment: ecosystems, communities, species, and genetic diversity. The main threat to biological diversity is human impact, manifested in the destruction of habitats, fragmentation, degradation, global climate change, and species overexploitation.

Among the flora of the Ukrainian Carpathians is the family *Ranunculaceae* Juss. deserves special attention. The family includes 102 species of plants that belong to 22 genera. Some members of the family are listed in the Ukrainian Red Book, which raises the critical question of preserving the biodiversity of these plants.

Delphinium elatum L., *Anemone nemorosa* L., and *Pulsatilla alba* Rchb. are especially worth mentioning among all species of the family. These plants have a long history of application in the folk medicine of Ukrainians and many other nations, thanks to their efficiency and wide range of activities. Therefore, these species may have invaluable potential for developing various new drugs.

D. elatum is a perennial herbaceous plant of the *Ranunculaceae* family 80-150 cm tall, common in the Ukrainian Carpathians (Marmaros Massif, Polonyna Borzhava, Chyvchyn, and Gorgan Mountains) within the Transcarpathian, Chernivtsi, and Ivano-Frankivsk regions. The plant is mentioned in Ukraine's Red Book. It is regarded as a rare plant as well [1, 2].

D. elatum has been used in ethnomedicine for a long time to treat diseases of central nervous system, and gastrointestinal tract, as a wound-healing, analgesic and anti-inflammatory agent. Alkaloids, hydrocarbons, carbonyl compounds, aromatic compounds, fatty acids, esters, mono- and sesquiterpenoids, and higher isoprenoids are contained in the plant [1, 2].

Anemone nemorosa L. is a perennial herbaceous plant of the *Ranunculaceae* family. Its average height is 15-25 cm. It is widespread in the forest zone of Ukraine (in the Ukrainian Carpathians, Podillya, and Polissya). However, the plant can be rarely found in the Left-Bank Forest-Steppe [1].

A. nemorosa is widely used in traditional medicine as an antitumour, anti-inflammatory, antispasmodic, sedative, diaphoretic, bactericidal, antimicrobial, antifungal, expectorant, and diuretic agent. The main biologically active substances of *A. nemorosa* are alkaloids, glycosides (protoanemonin, anemonin, ranunculine, some types of saponins, tannins), vitamin C, resins, organic acids (chelidonic acid), coumarins, flavonoids and γ-linolenic acid [1].

Pulsatilla alba Rchb. is a perennial herbaceous plant of the *Ranunculaceae* family, 15–30 cm tall. It is commonly found in the Ukrainian Carpathians (Chyvchyn Mountains, Marmarosky Alps, Chornohora, Svydovets) within the Transcarpathian and Ivano-Frankivsk regions [1].

P. alba is characterized by a high content of biologically active organic compounds, namely organic acids, traces of alkaloids, vitamins, resinous and tannins, about 20 different macro- and micronutrients, essential oils, γ-lactones, triterpenoids, sterols, chelidonic acid, coumarins. *P. alba* use in folk medicine as an antitumor, hypnotic, hypnotic, antifungal, antifungal [1].

For many years, folk medicine has made extensive use of these plants. These plants have mostly biological effects: anti-inflammatory, wound-healing, immunosuppressive, analgesic, antitumor, cardiotonic, antihypertensive, diuretic, antispasmodic, vasodilator and other. However, despite the wide range of pharmacological activities, the limited natural range does not allow the full use of these plants in the pharmaceutical industry. This is why it is important to find other ways to obtain a complex of biologically active compounds. One of the most relevant strategies is the culture of cells and tissues *in vitro* [3-7].

In order to repair damaged tissues, plants use non-differentiated callus cells. These cells can be grown *in vitro* for a variety of biotechnological uses. Small vacuoles and no chloroplasts for photosynthesis are features of callus cells. Due to these characteristics, they resemble non-differentiated meristemic cells.

Comparing cell culture techniques to traditional whole-plant cultivation, main benefits are as follows:

- cultured cells are not threatened by environmental factors (microorganisms or insects);
- cells of any plant, even rare or endangered ones, can easily be maintained to produce their secondary metabolites;
- the target bioactive compounds can be produced independently of external factors (e.g., soil composition or climate);
- secondary metabolite production can be automized by robotic systems, which decreases costs and efforts and improves productivity [8, 9].

Given the special conditions of their habitat and the complexity of cultivating these alpine species, we used a method that modern pharmaceutical science uses as an alternative method of obtaining biologically active substances – growing cells and tissues *in vitro*.

In vitro technology allows controlling the growth of plant cells and the accumulation of biologically active substances by optimizing the culture medium. That is why this method has advantages over procuring medicinal raw materials in nature and cultivating plants in special conditions.

The traditional methods of plant conservation and restoration are *ex-situ* methods. These methods mean the preservation of populations in the protected areas. However, current, up-to-date ways to preserve the biodiversity of flora include biotechnological methods. They aim to develop strategies for cultivating plants (especially endangered ones) *in vitro.*

Callus induction is a commonly used *in vitro* method to produce herbal medicines in the pharmaceutical industry. Callus induction results in the production of a callus, a tissue composed of dedifferentiated cells. These cells are characterized by constant disorganized growth and proliferation. It is noteworthy that the callus, subjected to various biotechnological treatments, may be able to produce secondary metabolites in amount that is not inferior to the quantitative content in plants. Thus, callus biomass can be considered to be a valuable raw material for the production of drugs.

The study aims to determine the potential of callus biomass of *Delphinium elatum, Anemone nemorosa,* and *Pulsatilla alba* to become an analog of medicinal plant raw material for the use in the pharmaceutical industry, to investigate the composition, antioxidant activity and antimicrobial activity of extracts of the callus biomass of the above-mentioned plants.

MATERIAL AND METHODS

Callus induction

The objects of the study are medicinal plant raw materials and callus biomass of *D. elatum, A. nemorosa*, and *P. alba* and extracts made on their basis (Figures 1-3).

Herbarium specimen of *D. elatum* (RD6321), *A. nemorosa* (RA3821), *P. alba* (RP8221), which served as study material, was collected in spring-summer of 2021 in the botanical garden of the Danylo Halytsky Lviv National Medical University (Lviv, Ukraine).

In previous studies, the optimal conditions for obtaining callus biomass *D. elatum, A. nemorosa*, and *P. alba* [10] were established. Explants were used to initiate callusogenesis segments of aseptically grown sprouts, namely roots, meristematic tips and hypocotyl. Cultivation

Figure 1.

Medicinal plant raw material *D. elatum*

Figure 2.

Medicinal plant raw material *A. nemorosa*

Figure 3.

Medicinal plant raw material *P. alba*

was carried out on Murashige-Skoog medium with growth regulators. The carbon source was glucose (30 g/l). Kinetin, 6-benzylaminopurine (BAP), 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthylacetic acid (NAA) in different concentrations were used for callusogenesis. The explants were cultivated under the following conditions: photoperiod 16/8 h (light/dark), illumination 2000 lux, temperature 25°C (±2°C), relative humidity 70%. Cultivation was conducted for 35–50 days.

The maximum biomass of *D. elatum*, (fresh weight = 48.03 g/100 ml and dry weight = 0.89 g/100 ml), *A. nemorosa* (fresh weight = 31.86 g/100 ml and dry weight = 1.67 g/100 ml), *P. alba* (fresh weight = 53.07 g/100 ml and dry weight = 2.76 g/100 ml) was registered in callus cultures grown *in vitro* under optimal conditions, which are presented in supplement table 1.

It was found that the maximum increase in callus biomass depended on the concentration and ratio of phytohormones and the type of explant. Callus cultures of *D. elatum, A. nemorosa*, and *P. alba*, obtained during the experiment, were used to obtain extracts and biologically active substances.

Extract preparation

Maceration was used to get extracts from the plant raw materials and callus biomass of *P. alba*, *A. nemorosa*, and *D. elatum* The plant raw material of *P. alba*, *A. nemorosa*., and *D. elatum* was aerial parts of plants, harvested in the flowering phase and dried at a temperature of 40–50°C. The callus mass of cells was dried on sheets of parchment paper at a temperature of 55±2°C. Dry biomass was used to obtain extracts. Once dry, the studied raw material was ground in a mechanical grinder to obtain a homogenous powder. As extractants, 20, 40, 70, and 90% concentrations of hydro-ethanolic solutions were utilised. For extraction, 10 g of each sample of dry crushed plant material was weighed and 200 ml of water-ethanol solutions of the appropriate concentration were added to each of them. For the extraction of the obtained callus mass, 3 g of dry crushed raw material of each sample was weighed and 60 ml of water-ethanol solutions of the appropriate concentration were added. The resulting extracts were stored for later analysis.

Phytochemical investigations

It was discovered that the resultant extracts included phenolic compounds and flavonoids, two physiologically active substances.

The Folin-Ciocalteu spectrophotometric technique was used to calculate the total amount of phenolic compounds [11]. The following ingredients were added to 1 ml of the extract 4 ml of a matching solvent (aqueous-ethanolic solution at a concentration of 20, 40, 70, and 90%), 0.5 ml of Folin-Ciocalteu reagent, 1.5 ml of 20% Na_2CO_3 solution. For 120 minutes, the mixture was maintained in a dark area. Then, the samples' optical densities were assessed using a Hitachi U-2810 spectrophotometer at a wavelength of 760 nm. Ethanol served as the control sample. All measurements are repeated three times, and the total amount of phenolic compounds is given as mg of gallic acid per 1 ml of extract.

Colorimetric analysis was used to determine the total flavonoid content [12]. 0.8 ml of a suitable solvent (aqueous-ethanolic solution at a concentration of 20, 40, 70, and 96 percent) and 1 ml of a 10 percent solution of $AlCl_3$ were combined with 0.2 ml of the extract. After 60 minutes, the mixture was removed. Then, the samples' optical densities were assessed, using a Hitachi U-2810 spectrophotometer at a wavelength of 420 nm. An $AlCl_3$ solution and ethanol mixture served as the control sample. All assays were repeated three times, and the overall flavonoid content was given as mg of quercetin per 1 ml of extract.

Antimicrobial activity

Using the agar diffusion method, the extracts of *D. elatum*, *A. nemorosa*, and P. alba's callus biomass were examined *in vitro* for their antibacterial and antifungal activity [13]. Eight clinical and reference strains of bacteria (Gram-negative, Gram-positive), and fungi have been tested with the extracts (tables 4–6). A 5.5 mm-diameter agar well was filled with 100 μl of the tested extract. Using a micrometer, the diameter of the growth retardation was determined with a 0.1 mm error. They utilized ethanol as a control.

Additionally, Petri dishes were incubated at 37°C for 24 hours for bacteria and at 25°C for 24–48 hours for fungi, using Mueller-Hinton agar and Saburo agar (for fungi). The MALDI TOF technique (Bruker, Bremen, Germany) and 16S rRNA gene sequences were used to previously identify reference and clinical microbial and fungal strains. All clinical strains had various antibiotic resistance patterns and were either extensively or multiple drug resistant. Clinical strains were isolated from a patient at a local hospital with healthcare-associated infections. Testing was done three times in total.

Antioxidant activity

Using a modified version of the Meda *et al.* approach, the antioxidant activity of plant extracts and callus biomass were studied [10]. The mixture of 1 ml of extract and 4 ml of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) ethanolic solution was left in a dark area for 30 minutes.

The samples' optical densities were then evaluated using a Hitachi U-2810 spectrophotometer at a wavelength of 517 nm, with ethanol used as the control sample. The formula calculates the antioxidant activity of the extracts:

$$\% \text{ inhibition} = [\frac{A_{Sample} - A_{Control}}{A_{Sample}}] \times 100\%$$

where $A_{control}$ – is the optical density of the original solution of DPPH, A_{sample} – is the optical density of the mixture of the extract with a solution of DPPH. All measurements were performed in triplicate.

The obtained results were statistically processed using the program STATISTICA 8 and the statistical functions of MS Excel. The arithmetic means value M, the arithmetic mean deviation m, the number

of repetitions n and the Student's t-test were determined [8].

Statistical analysis

Statistical data processing was performed using MS Excel 2019 software. Data were presented as arithmetic mean (M) and standard deviation (m). The reliability of the obtained data was evaluated using Student's t-test. The level of statistical significance was considered as $p<0.05$.

Ethical approval: The research conducted is not related to either human or animal use.

RESULTS AND DISCUSSION

The total concentration of flavonoids and phenolic compounds was determined in *D. elatum*, *A. nemorosa*, and *P. alba*. In addition, the extracts' antioxidant activity was also assessed. Tables 1–3 display the findings.

According to the data, the highest concentration of phenolic components, 2.4478 and 2.6230 mg of gallic acid per ml, was found in extracts of *D. elatum* callus biomass and plant raw materials, respectively, that were 40% hydro-ethanolic. The highest levels of flavonoids, 1.9883 and 2.0389 mg of quercetin per ml, were detected in extracts of *D. elatum* callus biomass and plant raw materials that were 40% hydro-ethanolic.

The most significant activity has 40% hydro-ethanolic extracts of callus biomass and plant raw materials, and the percentage of inhibition was 73, 172 and 77, 164, respectively, according to a study on the radical-absorbing activity of extracts derived from plant raw material and callus biomass of *D. elatum*.

The study's results indicate the presence of 40% hydro-ethanolic extracts of *D. elatum,* the maximum amount of phenolic compounds and flavonoids, which have high solubility in water and have a pronounced antioxidant effect. Therefore, the optimal extractant should be considered 40% hydro-ethanolic to obtain extracts with antioxidant properties.

The obtained results demonstrate that the greatest concentration of phenolic compounds, 4.4533 and 4.5221 mg of gallic acid per ml, was found in 70% hydro-ethanolic extracts of callus biomass and plant raw materials obtained from *A. nemorosa*. The maximum content of flavonoids was found in 70% hydro-ethanolic extracts of callus biomass and plant material of *A. nemorosa*, which was 3.8815 and 3.9734 mg of quercetin per ml, respectively.

The maximum activity is shown by extracts of callus biomass and plant raw materials that are 40% hydro-ethanolic, with the percentage of inhibition being 86.512 and 89.580, respectively. This was revealed by a study of the radical-absorbing activity of extracts obtained from plant raw materials and callus biomass of *A. nemorosa*.

Table 1.

Total content of phenolic compounds and flavonoids in extracts of callus biomass (CB) and plant raw material (PRM) from *Delphinium elatum* L. and antioxidant activity of corresponding extracts

	Studied hydro-ethanolic extract							
	20%		40%		70%		90%	
	CB	PRM	CB	PRM	CB	PRM	CB	PRM
Total content of phenols [mg of gallic acid in ml], $\bar{x} \pm \Delta\bar{x}$, $n = 3$	1.7880 ±0.03	1.8051±0.07	**2.4478±0.01**	**2.6230±0.08**	2.0096±0.02	2.1404±0.02	1.5467±0.03	1.6218±0.01
Total content of flavonoids [mg of quercetin in ml], $\bar{x} \pm \Delta\bar{x}$, $n = 3$	1.3705±0.01	1.4963±0.05	**1.9883±0.02**	**2.0389±0.06**	1.3761±0.04	1.4491±0.02	1.3356±0.07	1.3945±0.04
Antioxidant activity, DPPH [%], $\bar{x} \pm \Delta\bar{x}$, $n = 3$	40.749±0.09	44.573±0.03	**73.172±0.01**	**77.164±0.02**	70.047±0.05	74.345±0.08	61.087±0.02	65.810±0.03

Supplement table 1.

The optimal conditions for callus induction for *Delphinium elatum L., Anemone nemorosa L.*, and *Pulsatilla alba* Rchb.

		Scheme of pre-sowing treatment and surface sterilisation	Type of explant	The composition of the culture medium
Optimal conditions for callogenesis	*D. elatum*	1.Mechanical treatment by damaging the seed coat, 2. Soaking seeds with sterile, cold water for 48 h (temperature 4°C). 3. Soaking with 70% ethanol for 3 min. and then 10% NaClO for 20 min. 4. Washing three times in the sterilized water.	hypocotyl	MS+2,4-D(1.0)+BAP(10.0)
	A. nemorosa	1. Cold stratification (3-4°C) for 60 days. 2. Soaking seeds in gibberellic acid solution (0.1) for 24 hours (temperature 4°C). 3. Soaking in 98% ethanol for 8 minutes. and then 20% H_2O_2 for 10 min.	roots and hypocotyl	MS+2,4-D(0.5)+ kinetin(0.5)+NAA(0.6)
	P. alba	1. Cold stratification (3-4°C) for 60 days. 2. Soaking seeds in gibberellic acid solution (1.0) for 24 hours (temperature 4°C). 3. Soaking in 98% ethanol for 5 minutes. and then 30% H_2O_2 for 20 min.	meristematic tips and hypocotyl	MS+2,4-D(0.5)+NAA(1.5) kinetin(1.0)

MS – Basal MS medium (Murashige and Skoog 1962), 2,4-D – dichlorophenoxyacetic acid, BAP – 6-benzyloaminopurine, NAA – 1-naphthylacetic acid

Table 2.

Total content of phenolic compounds and flavonoids in extracts obtained from callus biomass (CB) and plant raw materials (PRM) of *Anemone nemorosa* L. and antioxidant activity of corresponding extracts

	Studied hydro-ethanolic extract							
	20%		40%		70%		90%	
	CB	PRM	CB	PRM	CB	PRM	CB	PRM
Total content of phenols [mg of gallic acid in ml], $\bar{x} \pm \Delta\bar{x}$, $n = 3$	2.8552±0.02	2.9155±0.01	3.3347±0.01	3.5482±0.05	**4.4533±0.02**	**4.5221±0.06**	3.9448±0.01	4.0132±0.04
Total content of flavonoids [mg of quercetin in ml], $\bar{x} \pm \Delta\bar{x}$, $n = 3$	2.4194±0.03	2.7757±0.03	3.0112±0.02	3.1128±0.01	**3.8815±0.07**	**3.9734±0.08**	3.5010±0.04	3.5873±0.03
Antioxidant activity, DPPH [%], $\bar{x} \pm \Delta\bar{x}$, $n = 3$	71.353±0.02	73.746±0.04	**86.512±0.06**	**89.580±0.05**	77.197±0.03	81.919±0.09	84.624±0.02	87.754±0.05

Table 3.

Total content of phenolic compounds and flavonoids in extracts obtained from callus biomass (CB) and plant raw materials (PRM) of *Pulsatilla alba* Rchb. and antioxidant activity of corresponding extracts

	Studied hydro-ethanolic extract							
	20%		40%		70%		90%	
	CB	PRM	CB	PRM	CB	PRM	CB	PRM
Total content of phenols [mg of gallic acid in ml], $\bar{x} \pm \Delta \bar{x}$, $n = 3$	3.4597±0.05	3.7526±0.02	2.6556±0.05	2.8120±0.08	**4.4397±0.02**	**4.8512±0.06**	3.1524±0.02	3.3733±0.09
Total content of flavonoids [mg of quercetin in ml], $\bar{x} \pm \Delta \bar{x}$, $n = 3$	3.0042±0.08	3.0956±0.04	2.0815±0.05	2.1485±0.09	**4.0234±0.07**	**4.1624±0.04**	2.9891±0.05	3.0911±0.01
Antioxidant activity, DPPH [%], $\bar{x} \pm \Delta \bar{x}$, $n = 3$	40.446±0.06	42.568±0.03	56.806±0.02	59.164±0.03	80.422±0.05	84.790±0.07	**85.049±0.06**	**88.945±0.06**

Table 4.

Antimicrobial activity of extracts obtained from *Delphinium elatum* L. (disc-diffusion agar method)

Microbial strains	Diameter of inhibition zone [mm]					
	90% extract		Control	70% extract		Control
	PRM	CB		PRM	CB	
Reference strains						
Staphylococcus aureus ATCC 25923 (F-49)	18.0±0.2*	18.0±0.3*	9.0±0.2	14.5±0.4*	14.0±0.3*	8.0±0.3
Staphylococcus epidermidis 191	16.0±0.3*	17.0±0.4*	9.0±0.2	15.0±0.2*	14.0±0.2*	8.0±0.6
Escherichia coli ATCC 25922	11.0±0.2	11.0±0.25	10.2±0.4	12.0±0.3	13.0±0.25	10.0±0.2
Bacillus licheniformis ВКПМ-7038	14.0±0.35	14.0±0.4	11.0±0.5	14.0±0.4	14.5±0.2	10.0±0.5
Pseudomonas aeruginosa ATCC 27853 (F-51)	15.0±0.3	15.0±0.3	9.0±0.6	12.7±0.3	13.0±0.3	8.0±0.3
Candida albicans ATCC 668-853	13.0±0.4*	14.0±0.3*	11.0±0.6	11.0±0.3*	11.0±0.2*	0
Candida albicans ATCC 885-653	13.0±0.3*	14.0±0.2*	11.0±0.4	11.0±0.2*	11.0±0.2*	0
Clinical strains						
Staphylococcus aureus №142	16.0±0.25*	16.0±0.3*	9.0±0.5	13.0±0.25*	13.0±0.2*	0
Escherichia coli №5	12.0±0.25	13.0±0.35	10.0±0.3	11.0±0.3	13.0±0.4	9.0±0.25
Proteus vulgaris 165	13.0±0.2	13.5±0.2	11.0±0.2	10.5±0.2	11.0±0.25	10.0±0.4
Candida albicans 117 (*ket*)	12.0±0.3	12.0±0.4	10.0±0.7	9.0±0.2*	10.0±0.2*	0
Candida albicans 60 (*nys*)	11.5±0.3	12.0±0.4	10.0±0.4	9.0±0.2*	10.0±0.3*	0

CB – callus biomass, PRM – plant raw material; *p<0,05 regarding control

Analysing the study results, we can assume that using a 70% water-ethanol mixture as an extractant we obtain an extract of *A. nemorosa* with the maximum flavonoids and phenolic compounds, which have low solubility in water but have more pronounced antimicrobial properties. The research results are presented in table 5.

Using a 40% water-ethanol mixture as an extractant, we will obtain an extract of *A. nemorosa* with phenolic compounds and flavonoids which are more soluble in water in smaller quantities but exhibit a more pronounced antioxidant effect. It should be noted that it is expedient to conduct further studies on determining the component composition of phenolic fractions of the studied extracts.

The obtained results indicate that the callus biomass and plant raw materials of *P. alba* used to make the hydro-ethanolic extracts had the highest concentration of phenolic compounds. The amounts of gallic acid in each ml were 4.4397 and 4.8512 mg, respectively. The highest concentration of flavonoids, 4.0234 and 4.1624 mg of quercetin per ml, respectively, was discovered in 70% hydro-ethanolic extracts of callus biomass and plant raw materials of *P. alba*.

40% extract		Control	20% extract		Control
PRM	CB		PRM	CB	
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
7.0±0.2*	7.0±0.4*	0	0	0	0
7.0±0.2*	7.5±0.4*	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0

The results of a study on the radical-absorbing capacity of extracts made from plant raw materials and callus biomass of *P. alba* revealed that extracts that are 90% hydro-ethanolic have the highest capacity, with respective inhibition percentages of 85.049 and 88.945.

Antimicrobial activity

The antimicrobial and antifungal activity of *D. elatum*, *A. nemorosa,* and *P. alba* extracts against referent and clinical microbial strains have been evaluated. The research results are presented in tables 4-6.

90.0% and 70.0% *D. elatum* extracts showed significant activity against Gram-positive microorganisms (clinical and reference strains of *S. aureus*, and reference strain *S. epidermidis* (tab. 4). In addition, 90.0%, 70.0% and 40.0% *D. elatum* extracts showed antifungal activity against clinical and reference strains of *C. albicans*, with the best activity of 70.0% extract (tab. 4).

One study investigated the antimicrobial activity of the ethanolic extract of *D. elatum* against a range of Gram-positive and Gram-negative bacteria and fungal strains. The extract showed significant antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* with MICs of 64 and 32 μg/ml [14].

Extracts of *A. nemorosa* didn't show activity against bacteria (tab. 5), but 40.0% extract showed antifungal activity against clinical and reference strains of *C. albicans* (tab. 5).

One study investigated the antimicrobial activity of the methanolic extract of *A. nemorosa* against a range of Gram-negative bacteria, *Vibrio anguillarum*. *V. anguillarum* [15].

90.0% extract of *P. alba* showed activity against reference *E. coli* and clinical strain of *P. vulgaris*. 90.0/70.0/40.0% of extracts showed significant activity against *B. licheniformis* (table 6). 70.0% extract showed significant antifungal activity against clinical and reference strains of *C. albicans* (table 6).

The phytochemical study of two types of raw materials, *D. elatum*, *A. nemorosa* and *P. alba,* shows that both in the extracts of medicinal plant raw materials and in the extracts of callus biomass of the studied plants, the content of phenolic compounds and flavonoids is almost equivalent and sufficient for its biomass.

The study of antioxidant activity results allows us to consider the extracts of callus biomass *Delphinium*, *A. nemorosa,* and *P. alba* in terms of effectiveness equivalent to extracts of medicinal plant raw materials.

Table 5.

Antimicrobial activity of extracts obtained from *Anemone nemorosa* L. (disc-diffusion agar method), $p<0,05$

Microbial strains	Diameter of the inhibition zone [mm]					
	90% extract		Control	70% extract		Control
	PRM	CB		PRM	CB	
Reference strains						
Staphylococcus aureus ATCC 25923 (F-49)	8.0±0.2	8.0±0.2	8.2±0.2	7.0±0.2	7.0±0.5	7.5±0.5
Staphylococcus epidermidis 191	8.0±0.2	8.0±0.2	8.3±0.2	7.5±0.2	7.2±0.5	7.0±0.5
Escherichia coli ATCC 25922	9.2±0.2	9.2±0.2	9.5±0.4	8.5±0.5	8.2±0.2	9.0±0.2
Bacillus licheniformis ВКПМ-7038	10.0±0.5	10.0±0.2	10.5±0.2	8.5±0.5	8.5±0.5	9.0±0.5
Pseudomonas aeruginosa ATCC 27853 (F-51)	9.0±0.2	9.0±0.2	9.5±0.2	7.0±0.5	7.2±0.2	7.5±0.5
Candida albicans ATCC 668-853	11.0±0.4	11.0±0.4	9.0±0.6	8.0±0.2	8.0±0.2	8.0±0.2
Candida albicans ATCC 885-653	11.0±0.3	11.0±0.2	9.0±0.4	8.0±0.2	8.0±0.2	8.0±0.2
Clinical strains						
Staphylococcus aureus №142	0	0	0	0	0	0
Escherichia coli №5	9.0±0.5	9.0±0.2	9.0±0.2	9.0±0.5	9.0±0.2	9.0±0.5
Proteus vulgaris 165	9.0±0.2	9.0±0.5	10.0±0.2	9.5±0.5	10.0±0.5	10.0±0.5
Candida albicans 117 (*ket*)	10.2±0.3	10.0±0.2	8.0±0.5	0	0	0
Candida albicans 60 (*nys*)	10.0±0.3	10.0±0.3	8.5±0.5	0	0	0

CB – callus biomass, PRM – plant raw material; *$p<0.05$ regarding control

Table 6.

Antimicrobial activity of extracts obtained from *Pulsatilla alba* Rchb. (disc-diffusion agar method), $p<0,05$

Microbial strains	Diameter of the inhibition zone [mm]					
	90% extract		Control	70% extract		Control
	PRM	CB		PRM	CB	
Reference strains						
Staphylococcus aureus ATCC 25923 (F-49)	9.0±0.2	9.0±0.3	8.5±0.2	8.5±0.4	8.0±0.3	7.8±0.3
Staphylococcus epidermidis 191	9.0±0.3	8.0±0.4	8.8±0.2	8.0±0.2	8.0±0.2	8.0±0.6
Escherichia coli ATCC 25922	15.5±0.2*	14.3±0.25*	10.0±0.4	13.0±0.5	12.5±0.7	10.2±0.2
Bacillus licheniformis ВКПМ-7038	18.0±0.25*	17.0±0.4*	11.5±0.5	17.0±0.4*	17.5±0.2*	10.5±0.5
Pseudomonas aeruginosa ATCC 27853 (F-51)	12.0±0.3	12.0±0.3	9.3±0.6	10.7±0.3	11.0±0.3	8.2±0.3
Candida albicans ATCC 668-853	12.0±0.4	12.0±0.3	10.0±0.6	14.0±0.3*	14.2±0.2*	8.0±0.2
Candida albicans ATCC 885-653	12.0±0.3	12.0±0.2	10.0±0.4	14.0±0.2*	13.5±0.2*	8.0±0.2
Clinical strains						
Staphylococcus aureus №142	0	0	0	0	0	0
Escherichia coli №5	12.5±0.3	13.5±0.5	10.5±0.3	11.0±0.3	11.5±0.4	9.6±0.25
Proteus vulgaris 165	14.5±0.5*	15.0±0.2*	11.3±0.2	10.5±0.3	11.0±0.5	10.2±0.4
Candida albicans 117 (*ket*)	11.2±0.3	11.0±0.2	9.0±0.5	9.0±0.2*	10.0±0.4*	0
Candida albicans 60 (*nys*)	11.0±0.3	11.0±0.3	9.5±0.5	9.0±0.2*	10.5±0.4*	0

CB – callus biomass, PRM – plant raw material; *$p<0,05$ regarding control

40% extract		Control	20% extract		Control
PRM	CB		PRM	CB	
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
9.0±0.2*	9.0±0.4*	0	0	0	0
9.0±0.2*	9.5±0.4*	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
7.0±0.2*	7.0±0.4*	0	0	0	0
7.0±0.2*	7.5±0.4*	0	0	0	0

40% extract		Control	20% extract		Control
PRM	CB		PRM	CB	
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
11.0±0.2*	11.0±0.3*	0	0	0	0
0	0	0	0	0	0
7.2±0.2	7.2±0.4	6.0±0.2	0	0	0
7.2±0.2	7.3±0.4	6.0±0.2	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0

The antimicrobial activity study for reference and clinical strains recorded the equivalent efficacy of plant raw materials and callus biomass of *D. elatum*, *A. nemorosa* and *P. alba*.

According to the literature analysis, compared to other species, *D. elatum*, *D. staphisagria*, *D. anthriscifolium* var. savatieri, *D. nuttallianum*, *D. anthriscifolium* var. majus, and *D. cardiopetalum* generated comparatively more novel compounds. For example, *D. elatum* contains formamide which is formed from C-19 aldehydes [16], in phytochemical analysis of the seeds of *D. elatum* cv. Pacific Giant, four new C19-diterpenoid alkaloids with antitumor activity were isolated, emphasizing the research prospects in this area [17].

Numerous therapeutic substances with anti-cancer, immunomodulatory, anti-inflammatory, antioxidant, and antimicrobial actions have been discovered in the *A. nemorosa* plant [18]. In our study, extracts from callus biomass also showed qualities similar to those of extracts from plant raw materials.

CONCLUSIONS

Based on results, it can be concluded that researching potential plants among the Ukrainian (Carpatian) flora's representatives is important for searching for new active compounds. Plant raw material of *D. elatum*, *A. nemorosa,* and *P. alba* were introduced into *in vitro* culture.

The optimal conditions for stratification and sterilization of seeds and cultivation of plants *in vitro* were presented. In addition, a comparative analysis of extracts obtained from the callus biomasses and plant raw materials concerning antibacterial and antioxidant potential, the content of compounds of phenolic nature, and flavonoids were conducted.

The maximum content of flavonoids and phenolic compounds was observed in 40% hydro-ethanolic extracts of callus biomass and plant raw materials of *D. elatum* 90.0% and 70.0% *D. elatum* extracts showed significant activity against Gram-positive microorganisms. 90.0/70.0/40.0% extracts showed significant activity against *Bacillus licheniformis*. 70.0 % extract showed significant antifungal activity against clinical and reference strains of *Candida albicans*.

To protect the biodiversity and natural environment of the plants, it was discovered that the callus biomass of *D. elatum*, *A. nemorosa*, and *P. alba* is comparable to plant raw materials in terms of phenolic compounds and flavonoids as well as antimicrobial activity. Summarizing experimental

results, it was proved that the callus biomass of *D. elatum*, *A. nemorosa,* and *P. alba* have potential as analogs of medicinal plant raw materials, both for the content of biologically active substances and biological activities.

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