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THE STUDY OF THE NATURAL SUBSTANCES OBTAINED FROM THE POPLAR BUDS AND THEIR USE FOR PROTECTION AGAINST THE ACTION OF IONIZING RADIATION

Anna Mechshanova, Vladilen Polyakov, Temenuzhka Radoykova

Currently, natural plant extracts, which include biologically active substances, are increasingly used to produce medicines and cosmetics.

In connection with the dangers of a radioecological crisis, special attention is paid to finding ways to protect against the effects of chronic exposure to low-intensity ionizing radiation in natural conditions. Currently, there is no ideal and safe radioprotective agent available, and we are seeing a great effort to find these agents from natural sources.

Poplar extract is possible for use as a radioprotective shield from γ-radiation. Samples of protective screens were made from poplar extract on paper and showed a significant radioprotective effect. Phenolic compounds and flavonoids are widely present in plants as a second metabolite and are considered for research depending on their benefits for human health, healing and preventing many disorders. The main biologically active properties of flavonoids include antioxidant, anti-inflammatory, antitumor, rejuvenating, antibacterial and viral, neuroprotective and radioprotective action.

***The aim of this work** was – the study of Flavonoids in an extract obtained from poplar buds and the possibility of their use for protection against radiation.*

***Materials and methods.** The object of research is the vegetative organs of poplar (buds). In the process of work, experimental studies were carried out on the extraction and separation of natural compounds, identification of flavonoids, and study of the chemical composition of biologically active complexes of poplar and preparations based on them.*

***Research results.** Data from these studies provide the identification of flavonoids by spectroscopy and quantification of flavonoids in poplar bud extract and can contribute to the optimization of radioprotection procedures. The main components found in the poplar buds dry extract are 2',6'-dihydroxy-4'-methoxychalcone – are 2',6'-dihydroxy-4'-methoxychalcone – 2.67 %, 3,4-dihydro-2',6'-dihydroxy-4'-methoxychalcone – 2.33 %, pinobaxin – 1.91 %, chrysin – 0.76 %, pinostrobin – 0.04 %, pinocembrin – 0.61 %, tectochrysin – 0.54 % and galangin – 0.18 % of dry material. The results showed that the power of the penetrating radiation decreases with increasing the thickness of the protective screen. The power of penetrating radiation decreased from 78 % at the layer of 0.5 mm to 10 % at 3 mm layer thickness. Further increasing the thickness of the protective screen (>3 mm), doesn't affect the dose rate.*

***Conclusions**. The composition of the poplar buds' ethanol extract was investigated. Samples of protective screens made on the basis of poplar extract on paper showed a significant radioprotective effect on low-intensity ionizing radiation*

***Keywords**: Populus balzamifera, extraction, flavonoids, UV-VIS spectroscopy, radioprotectors, γ-radiation, HPLC, HPLC-MS, poplar buds, ethanol*

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1. Introduction

Since ancient times, poplar buds have been used in folk medicine as an antimicrobial and sedative agent. A significant number of extractive substances (up to 54 %) in the buds of balsamic poplar is reported [1]. The main share of extractive substances is lipids – about 70 %; essential oils, flavonoids, and waxes also are found in the extract [1]. Essential oils and alcoholic extracts of poplar buds have pronounced antimicrobial properties superior to propolis extract and even eucalyptus oil [2]. According to traditional medicine recipes, they have long been used to treat bronchitis, tuberculosis, and rheumatism as a wound healing and anti-inflammatory agent [3]. Recently, the effect of plant substances on the growth characteristics of filamentous and yeast fungi was studied [4, 5]. The application of essential oils and plant extracts during the storage of grain, vegetable, and fruit crops to protect them from numerous pathogens, including fungi of the genus Fusarium, was reported [6]. The antimicrobial properties of poplar bud extracts are associated with the presence of phenol carboxylic acids and flavonoids in their composition [7].

The organic acids that make up the poplar extract have a great influence on the human body: malic, tartaric, citric, succinic, which could increase the alkaline reserve of the body and influence metabolic processes. Aromatic carboxylic and hydroxycarboxylic acids: benzoic, salicylic, and cinnamic are responsible for the anti-inflammatory effect [8]. The significant iodine content in poplar bud oil is of significant interest due to the possibility of treating thyroid diseases [9]. It is described that a herbal preparation containing a flavonoid fraction and

having antitumor, anti-inflammatory, wound-healing and bactericidal activity is competitive to the synthetic antioxidant ionol in its antioxidant activity [10].

The composition of phenylpropanoids in poplar buds depends on a number of factors, such as the type and shape of the poplar, the phase of development, and the place of growth.

The available literature data indicate the presence in the bark and buds of poplars (white, black, laurel, fragrant) phenol glucosides, phenol carboxylic acids (coffee, ferulic, hydroxycinnamic), flavonoids, tannins [11]. The most numerous group of natural phenolic compounds are flavonoids. It is known that poplar buds are characterized by the presence of flavanones, flavones, flavonols, etc. [12, 13].

The compounds pinostrobin, pinocembrin, chrysin, tectochrysin, apigenin, kaempferol, quercetin, myricetin, galangin, isalpinin, isorhamnetin, rhamnetin, 2,6-dihydroxy-4'-methoxychalcone and 4',6'-dihydroxychalcone was found in the buds of poplar balsamic [14]. Poplar buds also contain protocatechuic, gallic, transcinnamic, p-coumaric, ferulic, and caffeic acids [14].

In connection with the dangers of a radioecological crisis, special attention is paid to finding ways to protect against the effects of chronic exposure to low-intensity ionizing radiation in natural conditions. Traditional radioprotectors, with their short duration of action and high toxicity, have proven to be unsuitable for chronic irradiation. Studies conducted in various countries have shown that biologically active substances of natural origin can be used as protectors at chronic radiation exposure [15].

Such protective natural substances include adaptogens: phyto- and zoological preparations of traditional medicine (alkaloids, polysaccharides), mixtures of biologically active substances, zoo effectors, telephone (hematopoietic stimulants), estrogens (compounds of prolonged systemic action), immunomodulators that mobilize the body's overall resistance to diseases, including those caused by radiation damage [16].

Benefits of flavonoids in various cases such as cytoprotective properties, antioxidant and neutralizing effect of free radicals, antiviral and antibacterial effects, antitumor properties in cancer prevention and treatment, and their anti-inflammatory effects have been proven in various cellular and animal studies [17, 18]. It was demonstrated that the introduction of various flavonoids reduced the effect of ionizing radiation on living organisms [19].

Ionizing radiation leads to biological, physical, or chemical transformations, which are called the radiation effect. When biological tissue is irradiated, free radicals of organic matter are formed. The organic radical has an unpaired electron and is highly reactive. It has a large energy reserve, and can break chemical bonds, which always occurs in the interval between the formation of ion pairs and the formation of final chemical products. In addition, the biological effect of radiation is enhanced by the oxygen effect. The highly reactive product resulting from the interaction of a free radical with oxygen leads to the formation of new molecules in the irradiated system [20].

Due to the presence of flavonoids in poplar extract, it could be hypothesized the formation of long-lived free radicals, therefore, the antioxidant activity of the extract. Therefore, it was of interest to investigate the poplar extract as a radioprotective shield against γ-radiation.

Therefore, it is necessary to investigate the correct use of flavonoids to prevent possible damage before and after exposure to radiation hazards.

The aim of the study is to investigate the extract obtained from poplar buds and the possibility of its use for protection against ionizing radiation, as well as determination of the optimal timing for the collection of raw materials.

2. Planning (methodology) of research

The first step is obtaining ethanoic extract of poplar buds. The next step was the determination of the presence of flavonoids in the extract using UV-VIS spectroscopy, a quantitative determination of flavonoids by high-performance liquid chromatography. And using the extract as a radioprotector (Fig. 1).

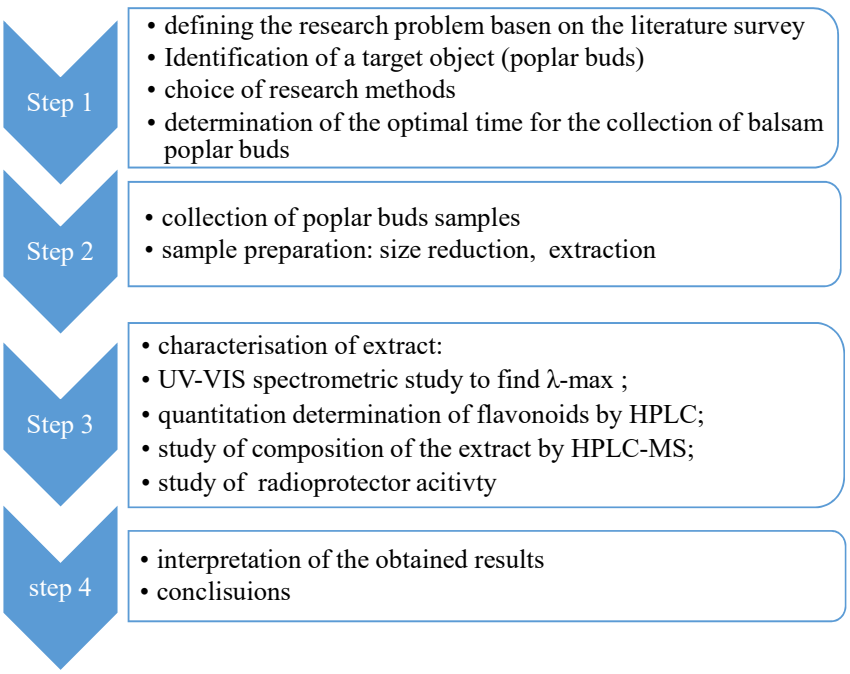

Fig. 1. Planning (methodology) of research

3. Materials and methods

3. 1. Sample preparation

Balsam poplar buds were collected in October–April 2021 in the vicinity of the village of Zarechny, North Kazakhstan region, Republic of Kazakhstan. Zarechnoye is a village in the Taiynshinsky district of the North Kazakhstan region of Kazakhstan. It is part of the

Mironovsky rural district. It is located about 6 km south of the city of Tayinsha, the administrative centre of the district, at an altitude of 174 meters above sea level. The geographical coordinates of the harvesting site are 53°46′16″ North 69°45′51″ East.

Storing poplar buds in sub-zero temperatures allows for saving the prepared raw materials for processing for a month. If storage is necessary, the best option is to store poplar buds at sub-zero temperatures with a layer thickness of no more than 15 cm. At temperatures of 0–10 °C, the layer should not exceed 5–10 cm, and the shelf life is 5 days. Storage in a thin layer (1.5–2 cm) at a temperature of 20–30 °C leads to drying. If these conditions are not observed, the buds become mouldy and become unsuitable for processing.

Accurate weighing of fresh poplar buds, crushed to particle size 2–5 mm, about 1.0 g was placed in a 150 ml flask with a thin section, 30 ml of 96 % ethanol was added, the flask was attached to a reflux condenser and heated in a water bath for 30 minutes. The flask was then cooled under running water to room temperature, and the contents of the flask were filtered through a paper filter into a 100 ml volumetric flask (solution A). Then, using the above method, extraction is repeated twice. The volume of the filtrate was adjusted to the mark of 96 % alcohol. The completeness of the extraction of flavonoids from raw materials was confirmed by a negative cyanidin test.

3. 2. Methods of Analysis

Determination of flavonoids content by UV-VIS spectrometry procedure.

5 ml of an alcohol extract from poplar buds (solution A) was placed in a 25 ml flask, 5 ml of a 5 % alcohol solution of aluminium chloride and 2–3 drops of diluted hydrochloric acid. The volume of the mixture was adjusted to the mark with 96 % ethyl alcohol. Time passes the reaction of complex formation in a place protected from light for 45 minutes.

To prepare a reference solution, 5 ml of an alcohol extract from poplar buds (solution A), 2–3 drops of diluted hydrochloric acid and brought the volume to the mark with 96 % ethanol.

The optical density of the resulting solution was measured on spectrophotometer Sf56 in the wavelength range 430 nm in a cuvette with a thickness layer 10 mm.

Calculations of the results.

To determine the entire contents of flavonoids spectrophotometric method was used, which is based on the ability of flavonoids to absorb in UV-VIS spectrum. The total flavonoids were determined using standard quercetin (GSO FS 42-1290-79), one of the most common flavonoids. In a similar investigation, the total phenolic compounds content was determined with a spectrophotometric method using standard gallic acid [21].

Preparation of a solution of GSO quercetin: about 0.05 g (accurately weighed) GSO quercetin, previously dried at a temperature of 130–135 °C for 3 hours, quantitatively transferred into a volumetric flask with a capacity of 50 cm^3, dissolved in 40 ml of 96 % aqueous alcohol when heated on the water bath. After dissolution, the contents of the flask are cooled to room temperature and brought to the mark with 96 % alcohol.

The content of the sum of flavonoids in terms of quercetin and dry raw materials was determined by the formula:

$$X = \frac{D * mq * 100 * 100 * 100 * 25}{Dq * m * 5 * (100 - w) * 50},$$

where D is the density of the test solution;

Dq – the optical density of a solution of a standard sample of quercetin;

m – the mass of raw materials in grams;

w – weight loss during drying of raw materials in % [22].

Quantitative determination of flavonoids in the obtained extracts by HPLC.

Flavonoids in poplar buds were analyzed by high-performance liquid chromatography (HPLC) on an Agilent 1100 liquid chromatograph with an Agilent 1100 Series Diode Array diode array detector, an autosampler, and ChemStation chromatographic data processing software. Chromatography conditions: column filled with ZORBAX Eclipse XDB-C8 reverse-phase sorbent, 4.6×150 mm. Temperature – 25 °C. Gradient elution in the system methanol – 0.1 % CF_3COOH (from 20 to 100 % methanol), eluent flow rate – 0.8 ml/min, sample volume – 2 µl, analytical wavelengths – 290, 326 nm.

Studying the composition of the extract by HPLC-MS.

HPLC/MS analysis was carried out using an Agilent 1100 Series LC/MSD liquid chromatograph with a diode array and mass-selective detectors. For the mass selective detector, the atmospheric pressure chemical ionization (APCI) method was used. Scanning of negative ions with m/z 100–700 with resolution m/z 0.1. Operating parameters for APCI: dryer gas flow (nitrogen) – 4 l/min with a temperature of 340 °C. The temperature of the evaporator is 400 °C. Separation was carried out on a column 4.6×150 mm filled with a reversed-phase sorbent Zorbax Rx-C18, 5 µm [23]. Qualitative analysis of individual compounds was carried out by comparing the retention times of the peaks obtained on the chromatograms of the analyzed samples with the retention times of the peaks obtained by elution of standard solutions of the analyzed substances.

Studying radioprotector activity.

When the solvent evaporates from the obtained extract, a thick substance is formed – a viscous, transparent greasy substance of brown colour. The study of this substance evaporated at room temperature (with 30 % yield) from poplar extract as a radioprotective shield against γ-radiation was carried out on an MKS-01R radiometer with a γ-radiation source power of 400 µSv/h. Ethyl alcohol, aluminium and copper foil were used as controls [24].

Methods of statistical data processing.

The results obtained were processed by the method of mathematical statistics [25]. All measurements were carried out at least in triplicate. Data are presented as mean±-

standard deviation. Statistical data processing is done with the help of software Microsoft Office Excel 2007.

4. Results

To determine the optimal time for the collection of balsam poplar buds, the collection of raw materials was carried out from October to April 2021. The results of the content of the total flavonoids in the ethanol extract of poplar buds in terms of quercetin and dry raw materials are presented in Table 1.

Table 1

The yield of flavonoids in the buds of poplar balsam

Month of sampling	Yield of flavonoids, %
October	8.10±0.03
November	9.20±0.03
December	10.43±0.03
January	9.31±0.01
February	8.93±0.01
March	10.62±0.03
April	7.81±0.01

The total amount of flavonoids in the studied balsam poplar buds was 7.8–10.6 % of dry raw materials. The highest content of flavonoids was found in the buds collected in March. This extract in liquid state was used for further research.

This group of substances is characterized by the presence of two maxima – winter and spring. Perhaps this is due to a slight decrease in the content of flavonoids in winter.

Fig. 2 shows that the UV-VIS spectra of the alcohol extract of balsam poplar buds have the main absorption maxima at a wavelength of about 290 and 326 nm. From the literature data, it is known that the absorption spectrum of most flavonoids is characterized by the presence of two main absorption maxima (bands) [26, 27]. One of them is located in the region of 320–385 nm (band I). Absorption in this region is due to the so-called cinnamoyl group present in the structure of the flavonoid molecule, which includes ring B and the adjacent part of ring C. Absorption in the region of 240–290 nm (band II) is due to the benzoyl group, which includes ring A and adjacent to him part of the ring C.

In addition, this region of the UV-VIS spectrum contains one of the absorption maxima of hydroxycinnamic acids, ferulic acid (λmax 291 and 323 nm), which are present in poplar buds. It is known that a solution of caffeic acid (λmax 299 and 326 nm) also found in an extract from poplar buds has a similar UV-VIS spectrum [28]. Comparison of the obtained results with the literature data allowed us to conclude that the nature of the absorption curve of extracts from poplar buds is determined mainly by substances of a flavonoid nature, the amount of which varies depending on the period of tree development.

The concentration of flavonoids in the studied extract was 0.0038±0.0001 g/mL in terms of quercetin.

Qualitative and quantitative analysis of evaporated ethanol extract of balsam poplar buds was performed by HPLC (Fig. 3). As analytical wavelengths, 290 and 326 nm were used, which were identified as the main ones in the UV-VIS spectrum of the balsam poplar ethanol extract (Fig. 3, *a*, *b*). Fig. 3 shows a chromatographic profile for the ethanol extract, the analysis of which makes it possible to identify the main group of signals with release times of 15.5–18.5 min. This fragment of the chromatogram and the UV spectra of the main identified compounds are shown in Fig. 4.

0.05 % solutions of flavonoid compounds in ethanol were used as standard solutions. Separated substances were identified by the method of internal normalization of peaks by comparing the peak and the retention time of solutions of standard samples (Table 2).

Quantitative determination of flavonoids in the thick extract was carried out by spectrophotometric method. We chose the reaction of the interaction of flavonoids with aluminium chloride as the basis of the analysis method in the environment of 96 % ethyl alcohol.

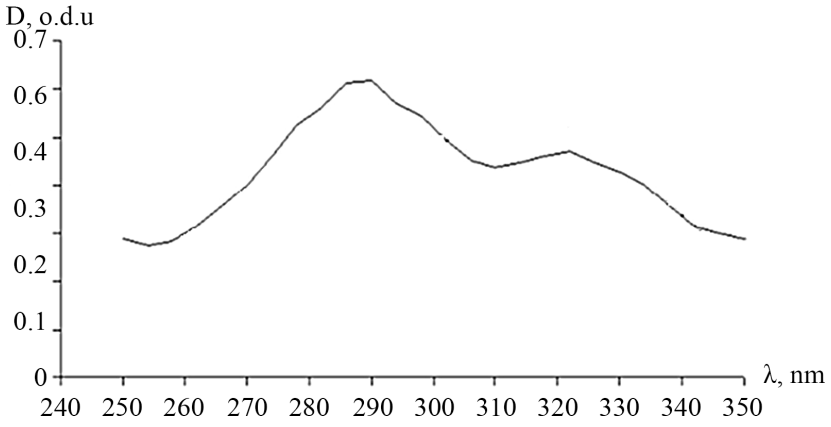

Fig. 2. UV-VIS spectrum of an alcohol extract balsamic poplar buds

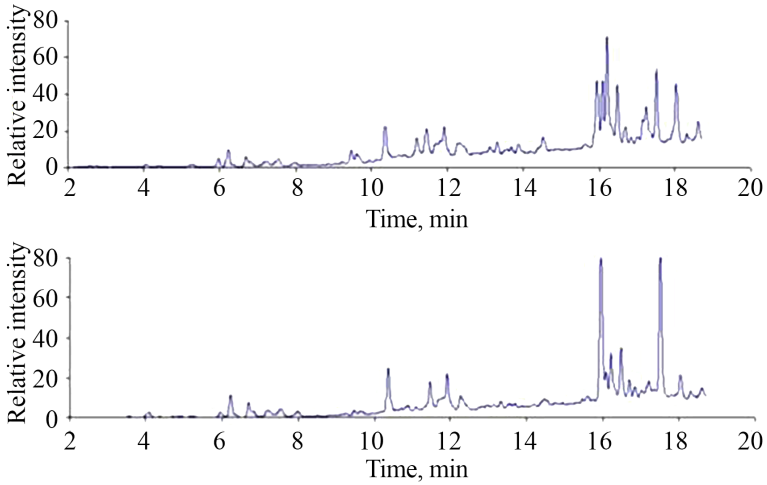

Fig. 3. HPLC chromatogram of a dry ethanol extract of balsam poplar buds: *a* – λ=290 nm; *b* – λ=326 nm

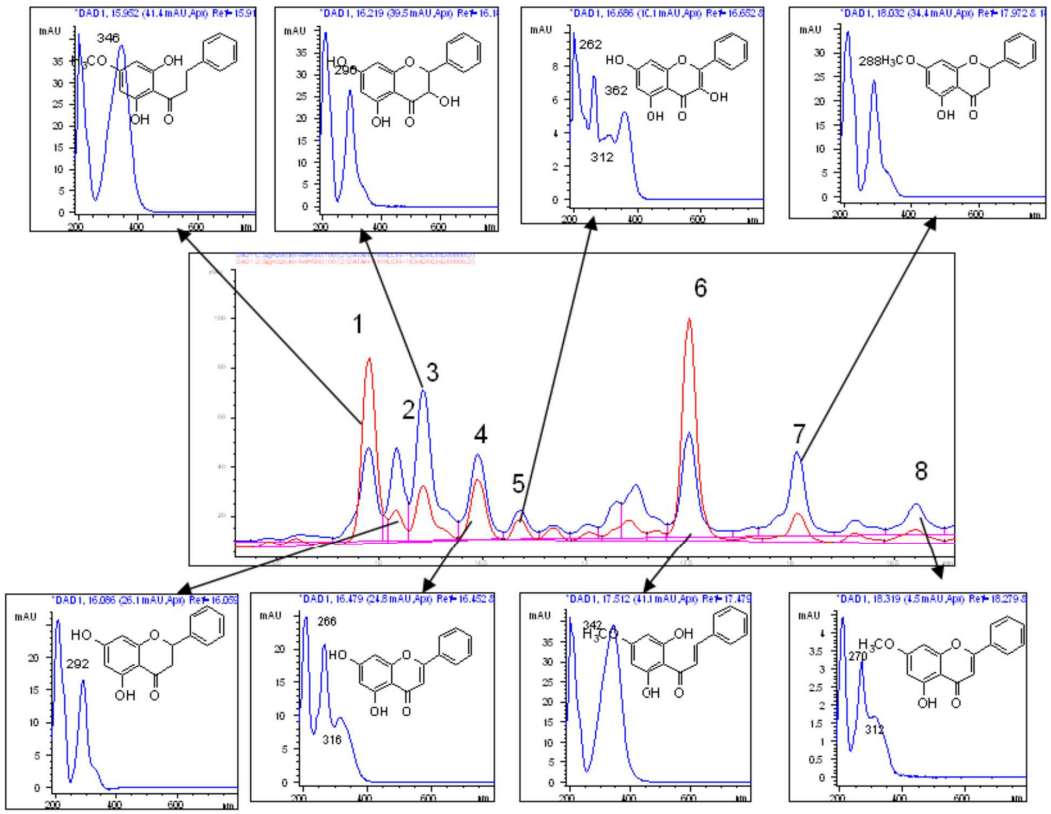

Fig. 4. A fragment of the HPLC-MS chromatogram of a dry ethanol extract of poplar buds in the range of 15.5–18.5 min and UV-VIS spectra of the main identified compounds

Table 2

Identified Flavonoids in poplar buds extract by HPLC/MS

Peak number on the chromatogram		1	2	3	4	5	6	7	8
Compound		3,4-dihydro-2',6'-dihydroxy-4'-methoxychalcone	Pinocembrin	Pinobaxin	Chrysin	Galantine	2',6'-dihydroxy-4'-methoxychalcone	Pinostrobin	Tectochrysin
m/z		72	56	72	54	70	70	70	68
Characteristic spectral ratios	A(280/254)	10.8	10.2	11.3	1.0	0.7	3.4	11.0	1,4
	A(320/280)	3.3	0.3	0.4	0.8	1.0	3.3	0.3	0,7
	A(360/254)	10.8	0.1	0.9	0.03	1.0	11.5	0.5	0,3
Retention time, min		15.95	16.09	16.21	16.48	16.68	17.51	18.03	18.32
Area, % (% from identified compound)		24.70	6.46	20.14	7.98	1.90	28.12	0.38	5.70
% of dry raw material		2.33	0.61	1.91	0.76	0.18	2.67	0.04	0.54
% of extract*10^{-2}		0.89	0.23	0.73	0.29	0.07	1.02	0.02	0.21

*Note: * – these results are suggested if all extracted compounds are registered on the chromatogram.*

It should be noted that the flavonoids identified in the extract of balsam poplar buds belong to different structural types, for which the values of characteristic spectral ratios were obtained (Table 2), which can be used for group identification of phenylpropanoids in extracts from plant materials [28].

The evaporated ethanol poplar bud extract (thick substance), which contains flavonoids, was studied as protective screens from γ-radiation. A thick substance was applied to the paper with a layer of 0.5–4.0 mm. As can be seen from Fig. 5, with an increase in the thickness of the protective screen, which is a poplar extract, the power of penetrating radiation decreases. If the protective screen increases more than 3 mm, the dose capacity remains constant.

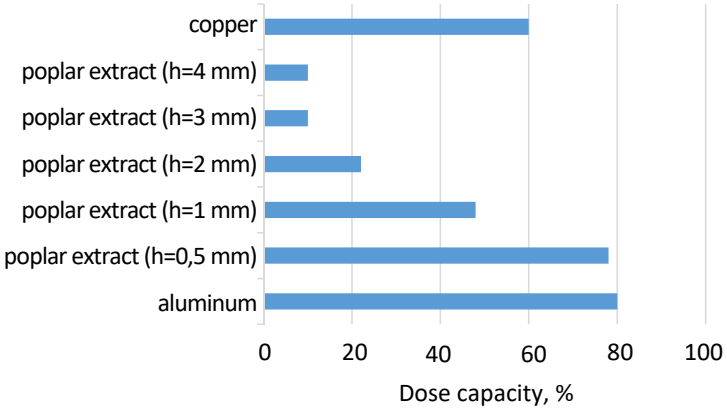

Fig. 5. Dependence of the dose rate of radiation on the parameters of the protective screen

Thus, it has been established that poplar extract can be used as radioprotective screens.

5. Discussion

Phenolic compounds are involved in the process of plant growth, acting as stimulants, and are formed most intensively in young, vigorously growing tissues, which include plant buds. According to the research results, the content of flavonoids in poplar buds increases with the beginning of the growing season (March).

According to the results of studies by other authors, it was found that the main group of alcohol compounds extracted are neutral substances, more than 60 % of which are acylglycerides, sterol esters. The alcoholic extract contained mainly flavonoids as substances with antimicrobial activity [29].

The composition of flavonoids of the balsam poplar buds growing in the Krasnoryak region contains tectochrysin, pinocembrin chalcones and significantly less pinostrobin. The main components are 2',6′-dihydroxy-4′-methoxychalcone, 3,4-dihydro-2',6′-dihydroxy-4′-methoxychalcone, pinobaxin and pinocembrin [6], which is comparable with the results obtained.

The results obtained are in good agreement with the data of other authors [30]. Thus, a comparative study of the buds of some poplar species showed that in all studied samples of the buds of Siberian poplar species and forms, there is a relatively low content of pinostrobin, but higher content of its chalcone counterpart compared to those in the buds of poplar species growing in the European part of Russia.

Works [31, 32] show the presence of poplar bud compounds such as apigenin, kaempferol, 7-methyl quercetin, and quercetin, which are part of the flavonoids the buds samples studied by us were not found.

More detailed information on balsam poplar bud flavonoids can only be obtained when studying the composition of flavonoids extracted by other solvents.

The results of the studies suggest that plant flavonoids, which were tested using the micronucleus test for anticlastogenic activity and the assay of thiobarbituric acid for antioxidant activity, do not show antioxidant activity in vitro, work as antioxidants in vivo, and their radioprotective effect may be associated with their activity at scavenging free radicals such as hydroxyl radicals. The anticlastogenic effects of 12 flavonoids and their antioxidant activity were investigated [33].

Flavonoids have shown promise for radioprotection and can be administered at higher doses with less toxicity. Research to reduce toxicity caused by ionizing radiation has focused on natural antioxidants. Free radical detoxification, control of inflammatory responses, and attenuation of apoptosis signalling pathways in radiosensitive organs are the main mechanisms of radiation protection and mitigation with flavonoids and natural antioxidants [34].

The study [35] demonstrates the ability of propolis to significantly reduce radiation-induced chromosome damage in human cells exposed to γ-rays in vitro. The closeness of the component composition of poplar and propolis buds was shown by researchers using modern chromatographic and spectral methods of analysis. According to the chemical composition, poplar buds are close to propolis, but the buds have a higher content of biologically active substances of a phenolic nature [36].

The protective effect of propolis against ionizing radiation can be explained as a direct removal of free radicals caused by an indirect action.

Biologically active substances of natural origin can be used as a method of protection against chronic exposure to low-intensity ionizing radiation. These substances increase the general nonspecific resistance of the body, stimulating the protective, antioxidant reserves of the body.

Study limitations. Analysis of the chromatographic and spectrometric determination of the component composition of the extracts was carried out by comparing the obtained spectra of the chromatographic peak with the spectra of reference compounds. Based on a comparison with the spectra of compounds presented in the database, not all substances were identified.

Prospects for further research. Poplar can be considered one of the promising sources of drugs for radiation protection. Further research will be aimed at creating an optimal technology for the synthesis of these drugs.

6. Conclusion

1. The optimal time for collecting balsam poplar buds was determined. The results of the content of the total flavonoids in the ethanol extract of poplar buds in terms of quercetin show that the content of flavonoids in poplar buds increases with the beginning of the growing season in March and amounts to 10.60 %.

2. The concentration of flavonoids in produced extract in terms of quercetin is determined and equal to 0.0038 g/ml.

3. The research results indicate that the main components are 2',6'-dihydroxy-4'-methoxychalcone – are 2',6'-dihydroxy-4'-methoxychalcone – 2.67 %, 3,4-dihydro-2',6'-dihydroxy-4'-methoxychalcone – 2.33 %, pinobaxin – 1.91 %, chrysin – 0.76 %, pinostrobin – 0.04 %, pinocembrin – 0.61 %, tectochrysin – 0.54 % and galangin – 0.18 % of dry material.

4. Around 70 % of the identified flavonoid in ethanol extract of poplar buds are2',6'-dihydroxy-4'-methoxychalcone, 3,4-dihydro-2',6'-dihydroxy-4'-methoxychalcone, pinobaxin.

5. The evaporated poplar extract was investigated as protective screens made based on paper showed a significant radioprotective effect. It was investigated that with an increase in the thickness of the protective screen, which is an extract of poplar, the power of penetrating radiation decreases. When applying poplar

extract 0.5 mm thick, the power of penetrating radiation is 78 %; when applying poplar extract 1 mm thick – 48 %.

Conflict of interests

The authors declare that there is no conflict of interest in relation to this paper, as well as the published research results, including the financial aspects of conducting the research, obtaining and using its results, as well as any non-financial personal relationships.

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Data availability

The manuscript has no associated data.

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Anna Mechshanova*, Doctoral Student, Department of "Chemistry and Chemical Technologies", Non-profit limited company "Manash Kozybayev North Kazakhstan University", Magzhan Zhumabaev str., 114, Petropavlovsk, Republic of Kazakhstan, 150010
ORCID: https://orcid.org/0000-0003-3407-2312

Vladilen Polyakov, Doctor of Chemical Sciences, Professor, Department of "Chemistry and Chemical Technologies", Non-profit limited company "Manash Kozybayev North Kazakhstan University", Magzhan Zhumabaev str., 114, Petropavlovsk, Republic of Kazakhstan, 150010
ORCID: https://orcid.org/0000-0003-4700-7560

Temenuzhka Radoykova, PhD, Assistant Professor, Department of Analytical Chemistry, University of Chemical Technology and Metallurgy, Kliment Ohridski blvd., 8, Sofia, Bulgaria, 1756
ORCID: https://orcid.org/0000-0002-2492-7359

***Corresponding author:** Anna Mechshanova, e-mail: Mechshanova_a@ptr.nis.edu.kz*