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## STUDYING THE QUALITATIVE COMPOSITION OF SUBSTANCES OBTAINED FROM POPLAR BUDS BY EXTRACTION AND BAROTHERMIC METHODS

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*Among the most acute problems of the Republic of Kazakhstan, it should be noted that the creation and development of a pharmaceutical base that meets all international standards, the development of the production of original domestic drugs, and the creation of safe and environmentally friendly technologies for their production. In this direction, plants of the genus Populus (poplar) of the Salicaceae (willow) family have an advantage due to large reserves of renewable raw materials (poplar plantations in the North Kazakhstan region have industrial reserves of medicinal raw materials) and the content of various classes of compounds with a wide range of biological activity. **The aim** of this work was to study the qualitative composition of substances from the buds of balsam poplar *Populus balsamifera* obtained by extraction and barothermal methods.*

**Objectives:** to obtain the substance from the balsam poplar buds *Populus balsamifera* by extraction and barothermal methods; establish the qualitative composition of the obtained substances; compare the composition of substances obtained by extraction and barothermal methods.

**Materials and methods.** Balsam poplar buds were collected in May 2021 near the village of Zarechny, North Kazakhstan region, Republic of Kazakhstan.

A method for obtaining a substance from balsam poplar buds includes using freshly harvested balsam poplar buds and extraction with solvents with an increasing polarity gradient. There were used solvents: hexane, dichloromethane, and ethyl acetate. The resulting extract was evaporated.

**The results and conclusions:** the results of the study showed the almost complete identity of the qualitative composition of the hexane extract of substances obtained by extraction and barothermal methods. In the case of ethyl acetate fractions, the difference is the presence of chalcones in the substance obtained by the barothermal method. Extraction with methylene chloride allows the separation of flavonoids, and subsequent extraction with ethyl acetate allows the separation of gibberellins

**Keywords:** *Populus balsamifera*, buds, pinostrobin, extraction, barothermic method, flavonoids, chalcones, chromatography, the composition of substances

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### 1. Introduction

One of the strategic priorities for the development of the Republic of Kazakhstan is to reduce the dependence of healthcare in Kazakhstan on imported medicines and, as a result, the use of their own resources of wild and cultivated raw materials and the creation of original herbal preparations based on it, not inferior in price and quality foreign counterparts.

Poplars, numbering more than 15 species in Kazakhstan, are interesting for their diversity, conservation and practical use. Poplar balsamic has a height of 25–30 meters; the trunk thickness is up to 2 meters, the bark of young trees is smooth, greyish-green, while that of mature trees is grey [1]. The buds are oval formations with a pointed top, sticky, reddish-brown, 1.5–2 cm long and 0.6–0.8 cm thick. Balsam poplar grows everywhere in Northern Kazakhstan [2, 3]. Some of its species have long been used in folk medicine for various diseases. Phenylglucoside salicin, a substitute for the well-known

anti-inflammatory drug ibuprofen, was found in many poplar species' inflorescences, leaves and bark [4]. The chemical composition includes such compounds: lipids, flavonoids, phenolcarboxylic acids, and tannins [5]. However, the chemical composition of poplar and the pharmacological properties of its components are still insufficiently studied [6].

A herbal preparation obtained from poplar buds, containing a flavonoid fraction and having antitumor, anti-inflammatory, wound healing and bactericidal activity, is not inferior in its antioxidant activity to the synthetic antioxidant ionol [7].

The production of medicines is one of the most important sectors of the national economy. Medicinal raw materials are used for the industrial production of pharmacologically active substances in an individual state, especially those whose synthesis is not yet feasible or economically inefficient, and also for their possible use as a substance in developing drugs with an even more

pronounced therapeutic effect [8]. One of the ways to search for new highly effective drugs among herbal substances is a systematic study of the experience of traditional medicine, the composition and pharmacological activity of herbal medicinal products used by it, and the isolation and study of the components that make up their composition [9]. The advantage of preparations based on biologically active substances of plants in many cases is obvious, since, on the one hand [10], there are practically no complications and undesirable side effects in their use, and on the other hand, there is a wide scope for manoeuvring, which is provided by a rich selection of plants with the same species [11].

From the buds of balsamic poplar, a number of effective preparations have been obtained that are not inferior and even superior in terms of the degree of action to drugs used in practice [12].

Since ancient times, poplar buds have been used in folk medicine as an antimicrobial and sedative [13]. The conducted studies showed a significant amount of extractive substances (up to 54 %) in the buds of balsamic poplar. The main share of extractive substances is lipids – about 70 %; essential oils, flavonoids, waxes, also pass into the extract [14].

According to studies by a number of scientists [15, 16], essential oils and alcoholic extracts of poplar buds have pronounced antimicrobial properties that are superior to those of propolis extract and, in some cases, eucalyptus oil. According to traditional medicine recipes, they have long been used to treat bronchitis, tuberculosis, and rheumatism, as a wound healing and anti-inflammatory agent [17].

Recently, several authors have been studying the effect of plant substances on the growth characteristics of filamentous and yeast fungi [18]. However, most studies have a pharmacological focus. At the same time, it is possible to use essential oils and plant extracts while storing grain, vegetable and fruit crops to protect them from numerous pathogens, including fungi of the genus *Fusarium* [19].

The antiseptic properties of poplar bud extracts are associated with phenolcarboxylic acids and flavonoids in their composition [20].

The organic acids that make up the poplar extract have a great influence on the human body: malic, tartaric, citric, and succinic, which have the ability to 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 [21].

Unsaturated fatty acids of poplar oil, especially such as linoleic, linolenic and arachidonic, play an important role in the metabolic processes of the human body. They cannot be synthesized; therefore, they are indispensable and must be supplied to the body from outside. Unsaturated fatty acids are part of cell membranes and other structural elements of tissues. They are involved in metabolic reactions, providing the growth process, normal structural functions, and capillary permeability, which is especially important in the course of

tissue processes. In addition, unsaturated fatty acids contribute to removing cholesterol from the body, thereby preventing the development of atherosclerosis [22].

The importance of the microelements of poplar oil is great. Zinc is an integral part of the hormone insulin. This element prevents inflammatory processes in the lung tissue, prostate gland, and organs of the genital area. Manganese is a part of enzyme systems and takes an active part in redox processes, affecting protein metabolism. Copper is involved in tissue respiration, hematopoietic processes, and the normal course of a number of neurological processes and stimulates the production of pituitary hormones. Cobalt plays an important role in the processes of hematopoiesis and is part of vitamin B12 [23].

Of particular interest is the significant iodine content in poplar bud oil, which leads to the conclusion that it can be used to treat thyroid diseases, the pathology associated with oncological diseases [24].

The class of organic compounds with a pyrone cycle in their structure is of interest in studying the mechanism of biochemical reactions and potentially physiologically active substances. In recent years, in the spectrum of biological activity, increased attention has been paid to antioxidant properties, so the question of an objective assessment of the inhibitory effect of the substances under study, which includes a large group of polyphenolic compounds (flavones, flavonols, chalcones, tannins) is relevant [25].

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 [26].

Thus, phenolic compounds were identified in poplar buds: *p*-hydroxyacetophenone, dimethylcaffeic acid, cinnamoyl-cinnamate, vanillin and many flavonoids - chrysin, chrysin-7-methyl ester, apigenin, galangin, 7-methylgalangin, 7-methylkaempferol, quercetin, 7-methylquercetin and 3,7-dimethyl quercetin, pinocembrin, 7-methyl-pinocembrin, 2,5-dihydroxy-7-methoxyflavanone and 2',6'-dihydroxy-4'-methoxychalcone [27].

In the buds of balsamic poplars growing in Northern Kazakhstan, the presence of such compounds as pinostrobin, pinocembrin, chrysin, tectochrysin was found in the composition of polyphenols. apigenin, kaempferol, quercetin, myricetin, galangin, isalpinin, isorhamnetin, rhamnetin, 2,6-dihydroxy-4'-methoxychalcone and 4',6'-dihydroxychalcone. Poplar buds also contain protocatechuic, gallic, transcinnamic, *p*-coumaric, ferulic, and caffeic acids [2].

When solving the problem of the practical use of extractive substances, it becomes necessary to determine the conditions for their maximum extraction. The search for optimal conditions for the process is one of the most common scientific and technical problems. The extraction process is influenced by various factors: extraction method, process hardware, type of extractant, its concentration, temperature and duration of the process, the ratio of raw materials and extractant, etc.

Establishing the role of factors that determine the extraction process is certainly important for the optimi-

zation of technological process in order to increase efficiency and increase the yield of finished products.

**The aim of the present research** was to study the qualitative composition of substances from poplar buds obtained by extraction and barothermal methods (SE and SB, respectively). Tasks: obtain the substance from the balsam poplar buds *Populus balsamifera* by extraction and barothermal methods; establish the qualitative composition of the obtained substances; compare the composition of substances obtained by extraction and barothermal methods.

## 2. Planning (methodology) of research

Fig. 1 shows a graphical representation of the research planning process.

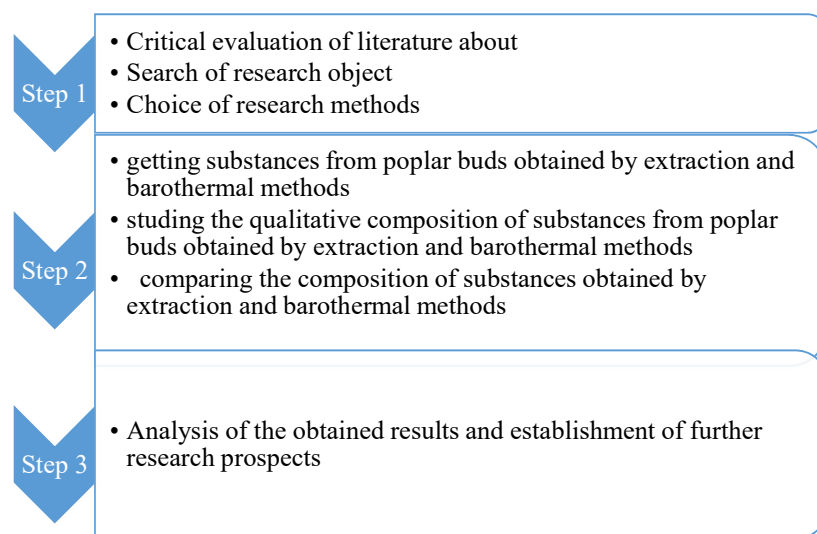


Fig. 1. Planning of the research

## 3. Materials and methods

To study the qualitative composition of substances from poplar buds, two different preparation methods were used: extraction and barothermal.

Balsam poplar buds were collected in May 2021 near the village of Zarechny, North Kazakhstan region, Republic of Kazakhstan. The buds were greenish-brown and brown, large, 12–23 mm long, strongly sticky, and aromatic.

**Extraction method.** The method for obtaining balsam poplar extract includes using fresh balsam poplar buds, grinding, and extraction with 90 % ethanol in a Soxhlet apparatus at a temperature of 60 °C for 24 hours, filtration and evaporation to obtain the target product (tar).

**Barothermic method.** This method consists of the fact that essential oil is extracted from poplar buds that have opened, under the influence of high temperature and high pressure, with superheated water vapour, which is then cooled, condensed and collected in a receiver. The extraction of the sum of substances from poplar buds by the barothermal method was carried out at a temperature of 140 °C under 10–15 mm Hg vacuum for one hour. The apparatus consists of a reactor (a 500 ml round-bottom flask), a collector (a 100–200 ml two-necked round-bottom flask with a built-in throat with a mesh filter), and upper and lower thermoelements (mantle heaters).

Determination of the qualitative composition of substances from poplar buds. To study the qualitative composition of the substance from poplar buds, solvents with an increasing polarity gradient were used:

- hexane (chemically pure)  $t_{bp}=68.7\text{ }^{\circ}\text{C}$ ,  $\varepsilon=0.009$ ;
- dichloromethane (chemically pure)  $t_{bp}=39.8\text{ }^{\circ}\text{C}$ ,  $\varepsilon=0.309$ ;
- ethyl acetate (chemically pure)  $t_{boil}=77.1\text{ }^{\circ}\text{C}$ ,  $\varepsilon=0.228$ .

Extraction with hexane substances from poplar buds obtained by extraction and barothermal methods. SB extraction was carried out with hexane ( $t_{bp}=68.7\text{ }^{\circ}\text{C}$ ). The resin weighing 1 g was extracted by heating to 60 °C for 20 minutes. After that, the resulting extract was cooled to room temperature.

The study of the substance obtained by the extraction method (SE) was carried out according to the same scheme (extraction of a resin weighing 1 gram with hexane on heating for 20 minutes, followed by decantation of the extract and its evaporation).

Identification of substances in the obtained extracts. Identification of classes of substances was carried out using the method of thin-layer chromatography.

Release of pinostrobin from a substance obtained from poplar buds. At the first stage of the process, we extract the substance with hexane when heated to 60 °C, then cool the resulting mixture and drain the extract. The second stage involves filtering the extract to separate the polymeric compound, in the case of SB. At the third stage of the process, the

filtrate is evaporated and upon standing, pinostrobin crystals precipitate from the resulting tar, which are washed off with petroleum ether at the fourth stage. The purity of the pinostrobin crystals thus obtained was determined chromatographically and by measuring the melting point.

Study of the qualitative composition of chloromethylene extract. The rest of the substance (SB) and (SE), with masses of 0.64 g and 0.95 g, respectively, is eluted with methylene chloride in a volume of 15 ml ( $t_{bp}=39.8\text{ }^{\circ}\text{C}$ ). We filter out the undissolved resin residues. Drain the filtrate and evaporate at room temperature.

Study of the ethyl acetate fraction of the substance. We extract the filter cake obtained by filtering the chloromethylene fraction with ethyl acetate to do this. Solvent  $t_{bp}=77.1\text{ }^{\circ}\text{C}$ , volume=15 ml, extraction time 2 hours. After that, drain the resulting extract.

Statistical data processing was carried out using Statistica 8.0 and Microsoft Excel 2010. The repeatability (convergence) was evaluated according to the results obtained under the same conditions in one laboratory (one performer, one piece of equipment) in a short period of time. Variance, standard deviation, and relative error were calculated. In the course of statistical processing, acceptable results.

#### 4. Research results

The aim of the study was to study the qualitative composition of the substance obtained by barothermal and extraction methods (SB and SE, respectively).

The scheme for separating the substance from poplar buds into groups of substances with a similar polarity gradient, using the indicated solvents, includes extraction of the resin with hexane, evaporation and obtaining a hexane fraction, then extraction with methylene chloride and evaporation to a chloromethylene fraction, subsequent extraction with ethyl acetate and evaporation to an ethyl acetate fraction.

In the course of sequential extraction, extracts were obtained, the further study of which was carried out by physicochemical methods.

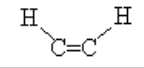
After extraction with hexane, the filtrate was evaporated, and the weight was 0.08 g and 0.05 g for SB and SE, respectively.

In the case of SB, the formation of a milky white suspension was observed within 10 minutes. The extract was poured off, and the resulting suspension was filtered off. On the filter, a dense, oily precipitate with a dense consistency was separated, the mass of which was 0.2 g per gram of poplar resin, i.e. twenty %.

Attempts to recrystallize this precipitate in non-polar solvents, particularly in petroleum ether, only led to the fact that the precipitate became lighter, but its complete dissolution did not occur. As a result, it was possible to separate the sediment from the ballast substances of the hexane fraction. The study of the precipitate obtained for solubility in solvents: ethyl acetate, methylene chloride, diethyl ether, ethyl alcohol showed its complete inertness; only swelling was observed. The foregoing suggests that the resulting precipitate is a macromolecular compound. To establish the nature of this substance, an IR spectrum was taken (Fig. 2). IR spectra of analyzed samples were registered using an IR-Fourier spectrometer FSM 1202 ("Infraspek", Russia).

When deciphering the spectrum, the peaks corresponding to the fragments of the molecules indicated below were highlighted (Table 1).

Table 1  
IR spectroscopy data for a macromolecular compound

Chemical bond	Vibration type	Frequency range, cm <sup>-1</sup> (literature data)	Value of identified vibrations, cm <sup>-1</sup>
-O-C=C-	Valence	1690–1635	1632, 1683
	Valence	725–675	698.4; 722.6
C=O conjugated esters	Valence	1730–1710	1704, 1727

Based on the given spectral data, it can be concluded that the resulting substance is a product of the polymerization of unsaturated carboxylic acids.

The above-considered precipitate (of polymeric nature) is formed only in the substance obtained by the barothermal method (SB). On the other hand, no polymeric compounds were found in the substance obtained by the extraction method (SE), which can be explained by the process of oxidation of unsaturated compounds (initial monomers) in the process of obtaining the substance (SE).

After the separation of substances of polymeric nature, we obtain a filtrate, in which, after two days, crystals of light yellow are formed. The crystals are filtered and washed with petroleum ether. The melting temperature of the obtained crystals of the substance was 96–98 °C, which did not give depression with a true sample of pinostrobin. Further, the resulting extracts of leaves were applied to chromatographic records. The determination was carried out in the system benzene – chloroform – formic acid – ethyl alcohol – diethyl ether (30:5:5:5:10) on Silufol plates.

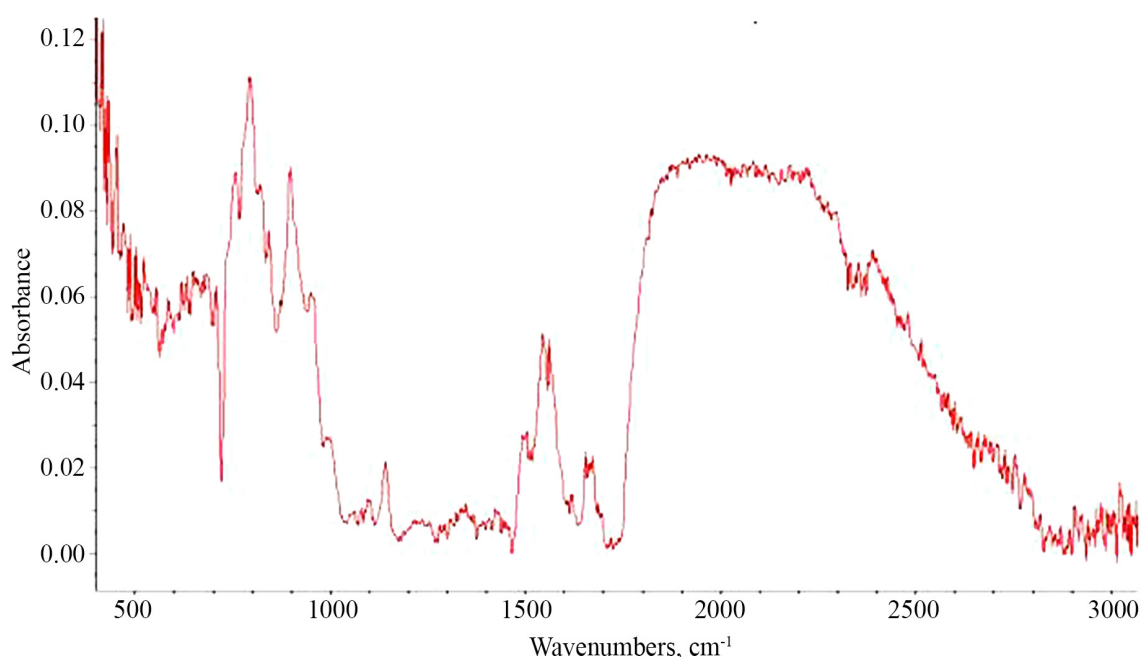


Fig. 2. IR spectrum of a macromolecular compound obtained by extraction with hexane of a substance obtained by a barothermal method

To the start line of a chromatographic plate previously activated in a dryer cabinet at a temperature of 100–105 °C, 0.02  $\mu\text{L}$  of the filtrate was applied with a micropipette. As substances-witnesses on the same plate was applied an alcohol solution of pinostrobin. The record was placed in a chromatographic chamber saturated with solvent vapours for 24 h and chromatographed in an ascending manner. After the solvent front passed about 8 cm, the plate was removed from the chromatographic chamber, dried, and the zones of substances were detected.

The resulting chromatogram was viewed in daylight, in UV light at  $\lambda=254\text{ nm}$  and  $\lambda=366\text{ nm}$ , and treated with a 3 % alcohol solution of aluminium chloride.

Therefore, the resulting crystals are pinostrobin. Recrystallization with ethyl acetate made it possible to obtain rather large crystals (Fig. 3). The studies were carried out using transmission electron microscopy on an EM-125K instrument.

The study of the substance (SE) testified that crystals of light yellow colour also formed in the obtained resin, which was washed with petroleum ether. Moreover, the melting temperature of these crystals also corresponded to the melting temperature range of pinostrobin, and the correspondence of these crystals to pinostrobin was also proved by comparative chromatography.

A chromatogram was obtained with crystals of the putative pinostrobin from substances (SB), (SE) and crystals of the pinostrobin marker, provided by the Research and Production Center Institute “Phytochemistry” (Fig. 4). The resulting spots have the same Rf value; hence they belong to the same substance, i.e. pinostrobin.

IR spectra were taken for crystals of the resulting pinostrobin (Fig. 5). IR spectra of analyzed samples were registered using an IR-Fourier spectrometer FSM 1202 (“Infraspek”, Russia).

Characteristic peaks are listed below (Table 2).

The reviewed literature sources [20] indicate that pinostrobin, like all flavonoids, is insoluble in non-polar solvents; however, in the course of the study, results were obtained indicating that pinostrobin is extracted with

hexane and forms crystals from a transparent homogeneous hexane extract.

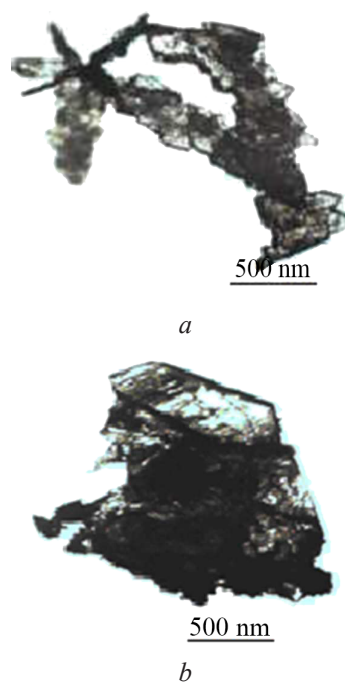


Fig. 3. Pinostrobin crystals: *a* – before recrystallization; *b* – after recrystallization

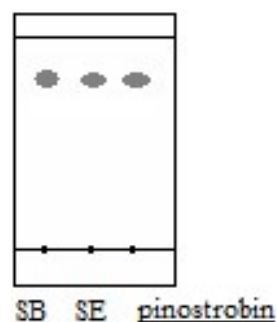


Fig. 4. Thin-layer chromatography of crystals obtained from the substance (SB), (SE) with a pinostrobin marker

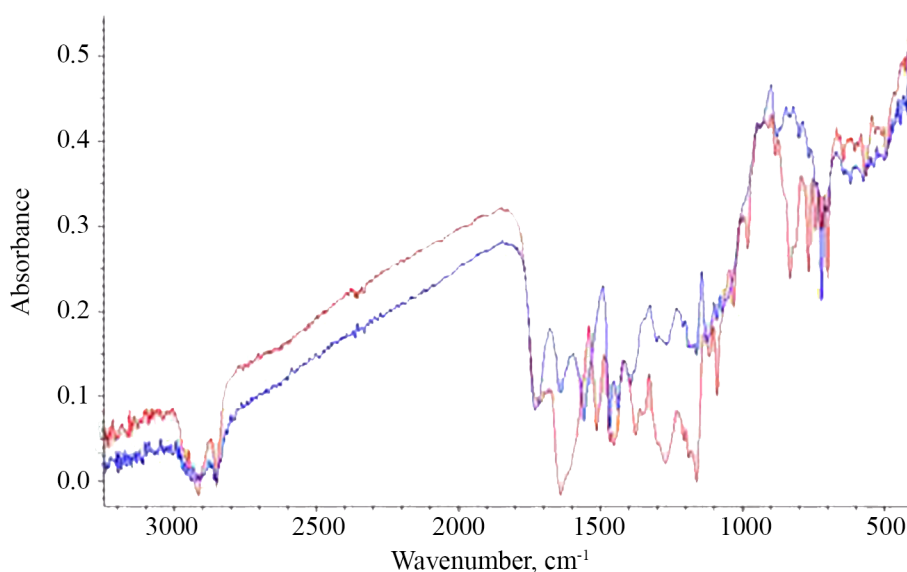


Fig. 5. IR spectrum of pinostrobin

Table 2  
Characteristic peaks of the IR spectrum of pinostrobin

Chemical bond	Vibration type	Frequency range, cm <sup>-1</sup> (literature data)	Value of identified vibrations, cm <sup>-1</sup>
Ar C-C	Valence	1625–1575	1620, 1574
C-O-C in saturated heterocycles	Valence	900–650	668, 888
C=O in saturated heterocycles	Valence	1525–1475	1505, 1471

The poplar hexane fraction washed off from the crystals was examined using thin-layer chromatography in the system petroleum ether – diethyl ether – acetic acid (80:20:1), with a pinostrobin marker, developed in iodine vapour and in ultraviolet light (Table 3).

Table 3  
Results of the chromatographic study hexane filtrate substance (SB) and (SE)

No. spot	<i>R<sub>f</sub></i> in literature	<i>R<sub>f</sub></i>		Assumed compound class
		SB	SE	
1	0.88	0.84	0.84	Wax
2	no	0.44	0.44	Pinostrobin
3	0.39	0.38	0.38	fatty acid
4	0.19	0.19	0.19	Sterols
5	0.15-0.21	0.13	0.13	Diglycerides
6	0.02	0.06	0.06	Monoglycerides
7	at the start	at the start	at the start	Phospholipids

On both tracks (SB) and (SE), a spot with an *R<sub>f</sub>* value corresponding to pinostrobin was observed.

Based on the results obtained, it can be concluded that the qualitative composition of the hexane extract of substances (SB) and (SE) is almost identical in quality. The only difference is the presence of a high molecular weight film-forming polymer formed upon extraction with hexane in the case of (SB) and its complete absence in the case of (SE). Therefore, it is assumed that this polymer is formed from unsaturated compounds that were preserved in obtaining the substance (SB) and oxidized during extraction in the case of (SE). The proof of this assumption is the fact that during the extraction with hexane of the resin (SR) stored for a long time with access to atmospheric oxygen, the amount of the polymer compound decreased from 20 % to 11 %; that is, the unsaturated compounds were oxidized.

We continue the study of the substance obtained in various ways according to the above scheme. In accordance with this, further elution is carried out with a solvent with a higher polarity gradient, which was chosen as methylene chloride.

The rest of the substance (SB) and (SE) were eluted with methylene chloride, the resin was separated, and its significant dissolution was observed. We filter out the undissolved resin residues. The filtrate is drained and evaporated at room temperature; the mass of the obtained resins for SB and SE was 0.8 g and 0.63 g, respectively.

Let us study the composition of the chlorostomethylene fraction filtrate by thin-layer chromatography. In the system, petroleum ether – ethyl acetate – acetic acid (4:2:0.1) on a Silufol plate. Inspection of the chromatogram showed the presence of a large number of spots. Spots were identified under the action of ultraviolet light. A schematic representation of the chromatographic plate is shown below (Fig. 6).

The *R<sub>f</sub>* values, colour, in visible light and under UV, of the detected spots are shown below (Table 4).

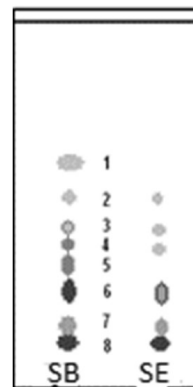


Fig. 6. Thin layer chromatography of chloromethylene substance extract (SB) and (SE)

Table 4  
Thin layer chromatography of chlorostomethylene extract of substances (SB) and (SE)

No. spot	<i>R<sub>f</sub></i>	Spot colour in visible light		Spot colour in UV		Assumed compound class
		SB	SE	SB	SE	
1	0.58	light yellow	light yellow	bright yellow	bright yellow	acids
2	0.47	light yellow	light yellow	dark brown	dark brown	acids
3	0.37	dark	light yellow	yellow	yellow	flavone
4	0.29	bright yellow	light yellow	dark brown	dark brown	flavonol
5	0.24	bright yellow	no	dark brown	no	flavonol
6	0.18	yellow-green	yellow-green	dark brown	dark brown	flavone
7	0.05	orange	orange	red-brown	red-brown	pigments
8	start	red-brown	red-brown	red-brown	red-brown	polyflavonoids

Some spots, in particular, 6, 5 and 7 (Fig. 6), had additional background colouration, which can be explained by the overlap of spots of two substances; in addition, spots 3, 4 and 5 had a very close *R<sub>f</sub>* value, which made identification difficult.

The subsequent study of the qualitative composition of the substance was continued by extraction with ethyl acetate of the filter cake obtained by filtering the chlorostomethylene fraction. After that, we drain the resulting extract; in the case of the substance (SE), it is dark

red, when, as in (SB), the colour is less saturated. The mass of the resulting resin is 0.04 g and 0.08 g for SB and SE, respectively. To compare the qualitative composition of both fractions, we use the thin-layer chromatography method on a Silufoll plate in the system benzene-chloroform-formic acid-ethyl alcohol-diethyl ether (30:5:5:5:10). A schematic representation of the resulting chromatogram is shown below (Fig. 7). The repeatability (convergence) was evaluated according to the results obtained under the same conditions in one laboratory (one performer, one equipment) in a short period of time. For each variance, standard deviation and relative error were calculated from three levels. In the course of statistical processing, acceptable results. With 9 independent definitions the method error did not exceed  $\pm 2.18\%$ .

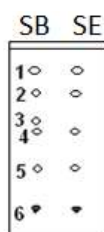


Fig. 7. Thin layer chromatography of the ethyl acetate fraction substances (SB) and (SE)

This chromatogram was examined under ordinary ultraviolet light ( $\lambda=365$  nm;  $\lambda=254$  nm), and was also treated with ammonia vapour, 5 % sodium carbonate solution, sulfuric acid in alcohol (70 % solution), sodium hydroxide in alcohol and phosphomolybdic acid (Table 5).

## 5. Discussion

Substances from balsam poplar buds were obtained from freshly harvested balsam poplar buds, and extraction was carried out with solvents with an increasing polarity gradient: hexane; dichloromethane; ethyl acetate, evaporation to obtain the desired product.

The results of the study showed an almost complete identity of the qualitative composition of the hexane extract of substances obtained by extraction and barothermal methods. In the case of ethyl acetate fractions, the difference is the presence of chalcones in the substance obtained by the barothermal method. Extraction with methylene chloride allows the separation of flavonoids, and subsequent extraction with ethyl acetate allows the separation of gibberellins.

**Study limitations.** The 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, and based on the comparison with the spectra from the database, the compounds whose spectra were in the registry were identified. Therefore, not all compounds, the quantitative content of which was determined by this method, could be identified.

**Prospects for further research.** The results obtained can be used to isolate pinostrobin from poplar buds. Further phytochemical and pharmacological studies of aerial parts of poplar and essential oil of poplar buds will be carried out. The obtained compounds can be used in agriculture and medicine.

Table 5  
Thin layer chromatogram of ethyl acetate extract substances (SB)

No. spot	R <sub>f</sub>	Spot colour					Assumed compound class
		H <sub>2</sub> SO <sub>4</sub>	NaOH (alc)	phosphomolybdic acid	UV <sub>365 nm</sub> +NH <sub>3</sub>	Na <sub>2</sub> CO <sub>3</sub>	
1	0.9	no	no	no	fluoresces	light yellow	phenolic acids
2	0.77	no	no	no	fluoresces	no	phenolic acids
3	0.58	no	red	light brown	red	light red	halkon
4	0.52	light yellow	yellow-green	blue	dark	yellow	coumarins
5	0.29	yellow	light red	light brown	no	no	gibberellins
6	0.03	red	red	brown	dark	dark red	polycondensed flavonoids

In terms of the number and colour of spots, the ethyl acetate fractions of the substance (SB) and (SE) are identical; the only difference is that in the case of (SB) the fraction has a spot (N° 3); this spot was absent.

When processing this chromatogram with a 70 % alcoholic solution of sulfuric acid, the spot with R<sub>f</sub>=0.29 showed a yellow glow in ultraviolet light; when this chromatogram was heated in an oven at  $t=120$  °C for 10 minutes, the spot began to fluoresce without changing colour, which indicates the presence of gibberellins, in particular, gibberellin A7.

## 6. Conclusion

Based on the results obtained, it can be concluded that the qualitative composition of the hexane extract of substances (SB) and (SE) is almost identical in quality. The only difference is the presence of a high molecular weight film-forming polymer formed upon extraction with hexane in the case of (SB) and its complete absence in the case of (SE).

In terms of the number and colour of spots, the ethyl acetate fractions of the substance (SB) and (SE) are identical, and the only difference is that in the case of (SB) the fraction has a spot (No); this spot was absent. In comparison with the works of other scientists, where it was found that the quantitative release of flavonoids is achieved by barothermic treatment of balsam poplar buds. In turn, extraction with supercritical carbon dioxide makes it possible to selectively extract pinostrobin with a high content in the extract [28].

Thus, it can be assumed that the scheme for studying the substance (SB) and (SE) makes it possible to separate flavonoids contained in poplar resin by extraction with methylene chloride, and subsequent extraction with ethyl acetate makes it possible to separate

gibberellins that stimulate plant growth. The qualitative composition of the substance (SB) and (SE) differs only in the presence of chalcones in the first and their absence in (SE).

#### Conflict of interests

The authors declare that they have no conflict of interest in relation to this research, whether financial,

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#### Data availability

The manuscript has no associated data.

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