Composition of Alcohol Extracts from Poplar Leaves (*Populus balsamifera* L.)

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The group composition of alcohol extracts from balsamic poplar leaves *Populus balsamifera* L, growing in the Krasnoyarsk Krai, Krasnoyarsk (Russia) were examined in this work. Leaves selected in different phases of the growing season were used: June, July, August – fresh, September – fallen leaves. Poplar leaves contain up to 56% of extractive substances, alcohol-soluble substances account for up to 67% of the extract amount. Alcohol extracts were fractionated using solvents with increasing polarity: petroleum ether (boiling point 40 to 70 °C), diethyl ether, ethyl acetate, butanol. The results showed that the alcohol extracts of poplar leaves are dominated by substances extracted with ethyl acetate (up to 60%). The study of the component composition of volatile compounds of individual fractions of alcohol extracts was performed on an Agilent chromatography-mass spectrometer. The presence of various groups of compounds was established: hydrocarbons, alcohols, acids, ketones, *etc*., including biologically active substances such as megasterol acetate, γ-sitosterol, sitosterol, 3,7,11,15-tetramethyl-2-hexadecene-1-ol, diethyltoluamide, 4-methoxy-3-nitrobiphenyl, and other compounds that it can be used in different applications.

DOI: 10.15376/biores.19.1.276-287

Keywords: Populus balsamifera; Leaves; Alcohol extracts; Fractionation; Group composition

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INTRODUCTION

The genus of *Populus* (Braslavsky 2012; Debbache-Benaida *et al*. 2013; Harbilas *et al*. 2013; Lee *et al*. 2019) is currently one of the most promising plant material sources for pharmacognostic research.

According to the FAO's International Commission on poplars, the total area of natural poplar plantations is more than 75.0 million ha, of which 96% are in Canada (30.3 million ha), Russia (24.8 million ha), and the USA (17.7 million ha) (FAO 2012). It should be noted that currently special attention is paid to the cultivation of poplar plantations. This cultivation normalises the balance between forest mass consumption and reproduction, as poplars are a fast-growing tree species. As of 2012, the total area of poplar plantations in the world was 8.6 million ha, of which 5.9 million ha (68.0%) were created for wood production and 2 million ha (23.0%) – for environmental purposes (FAO 2012).

There are more than 100 species of poplar, of which 30 species grow in the territory of the Russian Federation, whose central part is notable for about 20 species (Braslavsky 2012). According to the Unified Municipal Geoinformation System (EMGIS), the city of Krasnoyarsk (Russia) has 23,355 poplar trees (70% of all urban plantations), of which 17,390 are balsam poplar (*Populus balsamifera* L.), 5,120 are black poplar (*Populus nigra*

L.), 774 are silver poplar (*Populus alba* L.), and 71 are pyramid poplar (*Populus nigra* var. *italica* M.). The number of poplar trees in the city is 20% higher than coniferous species, such as spruce, pine, larch, and cedar.

To date, there are five pharmacopoeia types of poplar trees: black poplar, Canadian poplar, fragrant poplar, laurel-leafed poplar, and balsam poplar. As for pharmacopoeia raw materials (Emshanova 2018), only poplar buds are registered as such.

The use of poplar in the preparation of different medicines has been reported by a number of workers. Resin and essential oils produced from poplar buds, as well as tannins, organic acids, and poorly studied glycosides, are used in medicine, industrial perfumery, and aromatherapy. Ointments and tinctures obtained from poplar buds are used as anti-inflammatory, antipyretic, antiseptic, antifungal, antioxidant, and wound-healing agents for burns, infections, and purulent-inflammatory skin and soft tissue lesions. In terms of their chemical composition and pharmacological activity, medicines based on poplar buds are close to propolis (Braslavsky *et al*. 1991; Kurkin *et al*. 1994; Polyakov 1999; Teterev and Stupnikova 2001; Isaeva *et al*. 2008; Klishina and Nikitina 2010; Nikitina 2010a; Nikitina and Stepanyuk 2010b; Braslavsky 2012; Lee *et al*. 2019). It is known that balsam poplar (Canada) extracts and related salicortin can be used to reduce obesity and insulin resistance (Debbache-Benaida *et al*. 2013).

There has been a study (Dudonne *et al*. 2011) of antioxidant effect of phenolic compounds of *Populus nigra* bud extracts on skin aging. In Poland, in the field of dentistry, a study was conducted with regard to the anti-inflammatory effects of *Populus nigra* leaf bud extracts containing flavonoids (pinocembrine and pyrnostrobin) on human gingival fibroblasts (Pobłocka-Olech *et al*. 2019).

Leaves, contrastingly, have not been thoroughly studied and are mostly used in folk medicine as a remedy with antibacterial, analgesic, and soothing properties. Based on the leaves of *Populus tremula* (Lobanova 2012; Polyakov *et al*. 2012), medicines with anti-inflammatory properties were obtained.

It is known that the content of substances extracted with hot water in balsam poplar leaves is 1.3 times higher than that in buds, and almost twice as high as in branches (Khudorozhkova *et al*. 2007; Isaeva 2008; Naidenko *et al*. 2014; Isaeva *et al*. 2016a). The main group of water-soluble substances of the leaves are carbohydrates – 22.8% of the dry raw material (absolutely dry material) or ~ 66% of the dry extract substances (absolutely dry extract). In their composition, 55% are mono-, di-, and trisaccharides, 23% are colloidal polysaccharides (dextrins, easily hydrolysed polysaccharides, part of pectin substances, *etc.*), and 22% of the total carbohydrates is starch. The alcohol extract of the leaves also shows up to 5% carbohydrates (Naidenko *et al*. 2014).

The protein content in the aqueous leaf extract ranges from 5.8% (July) to 12% (August), and in the alcohol extract – 5% (absolutely dry material) (Isaeva *et al*. 2016a). The main protein components include aspartic and glutamic acids (19.8), leucine (9.3), and serine (6.2), essential amino acids – up to 33% of the total amino acids (Shepeleva and Isaeva 2014).

The leaves of black poplar contain simple phenols (salicin, populin), phenylpropanoids, tannins, vitamins C and E, carotenoids (neoxanthin, violaxanthin, lutein), and organic acids (Akulov 1986). Flavonoids were also found in poplar leaves, the highest content of which was found in the leaves of laurel-leafed and black poplar trees (1.6 and 4.1%, respectively). The leaves of balsam poplar show 2.9% of flavonoids (Kurkin and Kupriyanova 2020).

Thus, poplar leaves, along with buds, can be a prospective source of biologically active compounds. The study of the group composition of poplar leaves will help determine their medical properties and potential use, for example, in the treatment of various diseases. Therefore, the purpose of this research was to study the quantitative and qualitative composition of individual groups of alcohol-soluble substances of poplar leaves on the example of *Populus balsamifera* L., growing in the Krasnoyarsk Krai (Russia).

It should be noted that poplar leaves are an affordable and renewable type of raw material. The process of harvesting wood, annual sanitary pruning, and seasonal poplar fallen leaves results in a large amount of biomass, which is disposed of by incineration or taken to landfills in plastic bags. The study of the group composition of this type of waste will help to understand the directions of processing poplar leaves into valuable products, without causing irreparable harm to the environment.

It should be noted that previously the group composition of alcohol-soluble substances (including their volatile compounds) of balsamic poplar leaves growing in the Krasnoyarsk Krai, Krasnoyarsk (Russia), has not been studied.

EXPERIMENTAL

Materials

Current research was carried out to study the fresh and fallen leaves of balsam poplar (*Populus balsamifera* L.). Leaf samples were taken during 2018 to 2021: fresh (June, July, August) and fallen (September) leaves on the territory of Krasnoyarsk (Russia). The raw materials were dried, ground to a particle size of 5 to 6 mm, and stored in closed vessels at constant humidity.

Methods

Alcohol-soluble substances of plant substrates were removed by 3-h extraction with ethyl alcohol (96%), at the boiling point of the extractant. The ratio of material and extraction agent was 1:20 (weight of the sample was 10 g, and the moisture content of the raw material was 9.7%). The amounts of substances in the extracts were calculated by aliquot and expressed as a percentage relative to the mass of the absolutely dry sample (Ryazanova *et al*. 2012).

The substances contained in alcohol extracts were fractioned using solvents with increasing polarity: petroleum ether, diethyl ether, ethyl acetate, and butanol. The alcohol extracts were evaporated under vacuum. The residues were dissolved in hot water (2 mL), transferred to a separation funnel and then extracted with petroleum ether (boiling point 40 to 70 °C), diethyl ether, ethyl acetate, and butanol (Isaeva and Ryazanova 2016b). The obtained extracts were dried by adding a drying agent – anhydrous sodium sulfate, then evaporated under vacuum and re-dissolved in an appropriate solvent.

The fractions were qualitatively analysed using the Agilent 5975C-7890A chromatography-mass spectrometer by Agilent Technologies (Santa Clara, CA, USA, 2007) and an automatic sampler for Agilent 7683 liquid samples. A HP-5 column (copolymer 5%-diphenyl-95%-dimethylsiloxane) 30 m long with an inner diameter of 0.25 mm was used. Helium with a constant flow of 1.1 mL/min was used as a carrier gas. The column temperature was as follows: initial isothermal section 50 °C (2 min), temperature rise at a rate of 4 °C /min from 50 to 200 °C (0 min), and 20 °C /min to 280 °C (20 min).

The volume of the injected sample was 0.2 µL. The evaporator temperature was 280 °C, ionisation chamber temperature was 170 °C, and ionisation energy was 70 eV.

RESULTS AND DISCUSSION

Earlier, the authors found that the component composition of poplar leaves is represented by both low-molecular and high-molecular compounds. It should be noted that there is a large amount of extractive substances in the leaves, the content of which varies depending on the time of collection. It was found that during the development of the leaf, the content of extractive substances decreases from 56 (June) to 47% (August). The content of extractive substances in fallen leaves is 34% of the absolutely dry raw materials, while water-extractive substances predominate, accounting for about 86 %.

The alcohol-extractive substances of the leaves of the *Populus balsamifera* L., growing in the Krasnoyarsk (Russia) are poorly studied. Alcohol extraction makes it possible to extract various groups of chemical compounds, such as aromatic substances, flavonoids and a number of other biologically active compounds. They can be used independently, but in some cases, for example for bioconversion, their removal from the leaves increases the availability of the plant substrate for microorganisms during cultivation (Mamaeva and Isaeva 2021, 2022).

It is established that during the development of leaves, the content of alcohol-extractive substances decreases from 29 (June) to 5% (September, fallen leaves).

Table 1 shows the results of the group (fractional) composition of fresh (July) and fallen (September) leaves.

Table 1. Group Composition of Alcohol Extract of Poplar Leaves

Extract Name	Content (%)	
	Fresh Leaves	Fallen Leaves
Petroleum ether	1.2/11.1	1.2/15.0
Diethyl ether	0.1/3.8	0.4/12.2
Ethyl acetate	4.9/46.7	4.8/59.5
Butanol	3.1/28.7	1.7/20.3
Note – the numerator shows the content of absolutely dry material, the denominator – shows the total extractive substances; the relative standard error of the experiment does not exceed 5%		

Isaeva *et. al.* (2016a) previously worked on fractional estimation of poplar leaves selected in June and August, but this is the first study that has been conducted on fallen leaves also.

The obtained results show that the largest amount of substances from the ethanol extract of fresh (July) and fallen (September) poplar leaves was taken out with ethyl acetate (47 and 60%, respectively). It is known that flavonoids (Vedernikov *et al*. 2004; Galashkina *et al*. 2004) can pass into ethyl acetate. The content of these substances in Siberian poplar leaves is higher than that in the fresh leaves of balsam (1.7 times) and deltoid (2.3 times) poplar trees growing in Samara Oblast (Kurkin and Kuprieanova 2020). The content of this group of substances in poplar leaves decreases by the end of the vegetative period (Isaeva *et al*. 2016a). Butanol was used to extract glycosides of phenolic compounds acetylated with phenolic acids, acetic acid, or methoxylated by the carbohydrate part (Vedernikov *et al*. 2004). The share of these substances in fresh leaves (June to August) is 3 to 6% (absolutely dry material) (Isaeva *et al*. 2016a), in fallen leaves (September) to 2% (absolutely dry material).

The component composition of alcohol extract volatile fractions was studied using the example of fresh (months: June, July, August) and fallen leaves (September) using chromatography-mass spectrometry (GC-MS).

Table 2. Component Composition of the Petroleum Extract of Poplar Leaves

Retention Time (min)	Component	Content (% of the Total Substances)
7.764	1-ethylbutyl hydroperoxide	0.937
8.076	4-methyl-2-pentanol (methylisobutylcarbinol (MIBC))	1.206
29.071	diethyl phthalate	3.605
35.592	1.19-eicosadiene	0.920
35.751	6.10.14-trimethyl-2-pentadecanone	0.518
43.528	eicosane	1.762
42.316	3.7.11.15-tetramethyl-2-hexadecen-1-ol (phytol)	1.060
42.643	1-nonadecene	0.675
42.725	Z-14-nonacosane	0.750
42.778	2-cyclohexyl-undecane	0.659
43.180	1-(1.5-dimethylhexyl) -4-(4-methylpentyl)-cyclohexane	0.986
43.408	1.1'-[3-(2-cyclopent ylethyl)-1.5-pentanediyl]bis-cyclopentane	0.804
43.459	2-cyclohexyl-decane	0.853
43.636	1-(2.6-dihydroxy-4 -methoxyphenyl)-3-phenyl-. (E)- 2-propen-1-one	3.343
43.815	3.5-bis(1.1-dimethylethyl)-4-hydroxy-2.4-cyclohexadien-1-one	0.715
43.950	2-(acetoxymethyl)-3-(methoxycarbonyl)biphenylene	0.680
44.116	pentacosane	3.889
44.325	diethyl bis(trimethylsilyl) ester silicic acid	1.597
45.366	2.3-dihydro-6-nitro-1.4-phthalazinedione	0.703
44.477	diisooctyl ester 1.2-benzenedicarboxylic acid	2.877
44.658	2.4-dimethyl-benzo[h]quinoline	2.65
44.990	hexamethylcyclotrisiloxane	1.455
45.429	octadecane	3.083
45.578	1.4-phenylenebis[trimethyl]silane	0.725
46.011	Trimethyl(4-tert-butylphenoxy)silane	1.397
46.227	1-(4.4.4-trifluoro-1.3-dithiobutyl)-2-(3.3.3-trifluoro-1.2-dithiopropyl)-ethane	1.957
46.923	megestrol acetate	1.411
47.181	3-methyl-heneicosane	3.047
49.585	1.2-bis(trimethylsilyl)benzene	1.167
49.708	trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-silane	0.533
54.354	γ-sitosterol	5.135
Total identified		51.1

Table 3. Component Composition of the Diethyl Extract of Poplar Leaves

Retention Time (min)	Component	Content (% of the Total Substances)
7.774	3-hexanol (ethylpropylcarbinol)	18.060
8.132	2,4-dimethyl-2,4-pentanediol	49,689
19.245	1,1'-[(1-methylethylidene)bis(oxy)]bis-butane	1.971
29.073	diethylphthalate	4.628
42.905	4-dodecyl dimethyl ester 1,2,4-benzenetricarboxylic acid	0.824
43.030	1,2-bis(trimethylsilyl)benzene	0.600
44.120	hexamethylcyclotrisiloxane	2.080
43.474	decamethyltetrasiloxane	0.770
43.608	3,5-b is-trimethylsilyl-2,4,6-cycloheptatrien-1-one	1.130
44.483	5-methyl-2-trimethylsilyloxy-acetophenone	1.103
Total identified		80.9

Table 4. Component Composition of the Ethyl Acetate Extract of Poplar Leaves

Retention Time (min)	Component	Content (% of the Total Substances)
6.502	8a-ethoxy-3a,3b,7a,8a-tetrahydro-2,2,5,5-tetramethyl-, (8aR)-7H-1,3-dioxolo[4,5]furo[3,2-d][1,3]dioxin	0.555
7.760	3-hexanol (ethylpropylcarbinol)	1.562
8.078	1,5-dimethoxy-2,4-dimethyl-3-hexanol	2.571
8.259	4-ethyl-3-hexanol	0.517
9.554	monohydrate adipic dihydroxamic acid	1.182
9.655	N-acetyl-glycine	1.077
42.419	2-ethylacridine	0.564
42.728	trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-silane	0.669
43.315	3,5-bis-trimethylsilyl-2,4,6-cycloheptatrien-1-one	1.725
43.364	3,5-bis(1,1-dimethylethyl)-1,2-benzenediol	0.973
43.471	decamethyltetrasiloxane	1.950
43.539	1,4-phenylenebis[trimethyl]silane	1.782
43.680	3,5-bis-trimethylsilyl-2,4,6-cycloheptatrien-1-one	2.410
44.125	4-dodecyl dimethyl ester 1,2,4-Benzenetricarboxylic acid	1.820
44.852	hexamethylcyclotrisiloxane	2.700
44.224	methyltris(trimethylsiloxy)silane	1.013
44.242	1,2-bis(trimethylsilyl)benzene	1.078
44.320	Diethyl-1-(carb-n-butoxy)propylphosphonate	1.929
44.949	2,4-dimethyl-benzo[h]quinoline	3.260
45.285	1,1,1,3,5,5,5-heptamethyltrisiloxane	5.639
46.239	4-Methyl-2-trimethylsilyloxy-acetophenone	1.650
Total identified		36.6

As a result of the studied petroleum extracts (PE) of leaves of different development phases, the authors identified 118 (June), 169 (July), 144 (August), and 105 (September) components, diethyl extracts (DE) – 57 (June), 19 (July), 95 (August), and 55 (September) components, ethyl acetate extracts (EA) – 86 (June), 96 (July), 107 (August), and 51 (September) components.

Tables 2 through 4 show the component composition of the identified compounds of poplar leaves using (the content of which is higher than 0.5%) the example of the samples taken in July.

It was found that within the composition of petroleum extracts, from 30 to 80% is accounted for by the proportion of oxygen-containing compounds from the amount of identified substances (Fig. 1).

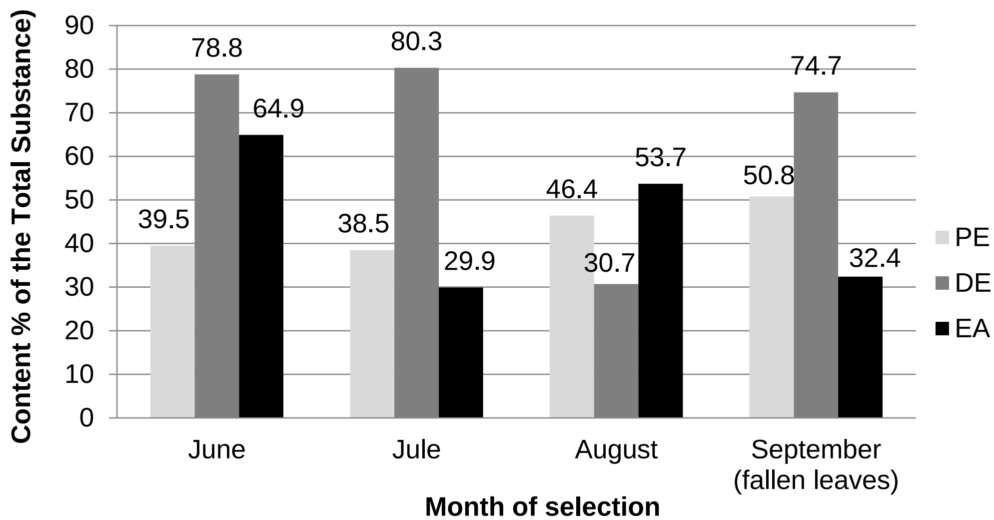

Fig. 1. Dynamics of the oxygen-containing substances in poplar leaves

Petroleum ether is known to extract terpenoids, n-alkanes, fatty acids, green pigments, and other plant substances. The main oxygen-containing compounds of the extracts of both fresh and fallen leaves are acid esters (June – 51.2%, August – 49.9%, and September – 58.7%). In the petroleum extracts of leaves selected in July, most of the compounds are ketones (up to 52.0% of the amount of oxygen-containing substances).

Silicon dioxide was also found (from 1.0 to 21.0%, depending on the month and solvent). The presence of silicon in poplar leaves was also confirmed by the results of a study of the elemental composition by inductively coupled plasma mass spectrometry (ICP-MS) on an Agilent-7900 mass spectrometer with argon plasma. Sample preparation of the samples consisted in their mineralization with a mixture of H_2O, HNO_3, H_2SO_4 (in a ratio of 1:1:1 by volume). The samples were an aqueous solution and were injected into the plasma without dilution in the form of a dry aerosol by means of an autoclave and a concentric sprayer. The carrier gas is argon. It was found that the silicon content in the leaves ranged from 0.4 to 0.8 mg/kg.

It is known that silicon is a number of biological properties, one of which is suppression of the growth of pathogenic microorganisms. The resistance of plants to damage caused by various pests increases with the increased content of silicon in them (Kolesnikov 2001).

Among the oxygen-free compounds in the petroleum extract of leaves, hydrocarbons from C_{20} and above were found, in amounts of: in June – 8.1%, July – 10.4%, August – 23.8%, and September – 1.2% of the total hydrocarbons). Among them, eicosanes were the main ones. Bromine-, fluorine-, sulfur-, and nitrogen-containing compounds were also found in the petroleum extracts.

It should be noted that 3,7,11,15-tetramethyl-2-hexadecene-1-ol (phytol) was found in the extracts of both fresh and fallen leaves. Phytol is one of the main acyclic monounsaturated diterpene alcohols. It is a part of chlorophyll and is a precursor of vitamins E and K. It is known from literature that phytol has anxiolytic (antiphobic), modulating metabolism, cytotoxic, antioxidant, inducing autophagy, and apoptosis, antinociceptive, anti-inflammatory, immunomodulatory, and antimicrobial effects (Islam *et al*. 2018).

The petroleum extract of fresh leaves selected in July showed the substances megestrol acetate (1.4%) and γ-sitosterol (5.1%); in September – 22.2-dihydrostigmasterol (sitosterol) (8.2% of the total identified substances). Megestrol acetate is part drug that is used to treat breast and endometrial cancer and other diseases (Kuhl 2005; Barakat *et al*. 2009; Yang *et al*. 2020). Sitosterol is a plant sterol that chemically resembles cholesterol. γ-Sitosterol is an important plant sterol and a biologically active compound. Reports (Sundarraj *et al*. 2012; Tripathi *et al*. 2013) show that it can be used as a component of anticancer medicines and to reduce cholesterol levels in blood.

It is known that esters and alcohols, partially glycolipids and phenolic compounds are extracted with diethyl ether. The diethyl extract of fresh leaves (July) shows a considerable share of alcohols: 3-hexanol and 2,4-dimethyl-2,4-pentanediol (68.8 % in total). Other extracts (August and September) show the share of alcohols of 7.9 and 31.8%, respectively, of the total the identified compounds. This amounts to 84.4 (July), 25.9 (August) and 42.0% (September) of the amount of oxygen-containing compounds.

It was also found that substances containing bromine and nitrogen are also present in diethyl extracts.

Ethylene brassylate (July and August) and 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[g]-2-benzo-piran (June) were found in the diethyl leaf extracts. They are notable for a strong and persistent musky odour and are used in perfumery. The diethyl extract of fallen leaves shows diethyltoluamide, a compound with repellent and insecticidal properties (Katz 2008).

Among the oxygen-free components, the main ones are short-chain hydrocarbons (C_{15} and below). The only exception is the diethyl extract of leaves selected in June, where 3-methylgeneicosan was found (36.3% of the total hydrocarbons).

The ethyl acetate extract of fresh leaves shows a high content of acids (crotonic and 2-butenic (E)-acids) of 32.4% (June) and 43.8% (August) from the total oxygen-containing compounds.

Among the oxygen-containing compounds, there is 8.1% of galaxoid (a polycyclic musky compound) (June) and ethylene brassylate – 13.4 (June) and 5.2% (August) from the sum of oxygen-containing compounds). In addition, the extract (August) shows hedion (3-oxo-2-pentenylcyclopentanacetic acid methyl ester) and β-iso-methylionone, which are also used in fragrance components and perfume compositions (Voytkevich 1994).

The June extract contains 4-methoxy-3-nitrobiphenyl, which is used as an important intermediate in the synthesis of bifenazate recognised as an effective bactericide (Chao *et al*. 2012).

In addition, it was found that sulfur-, nitrogen, phosphorus- and chlorine- (June) containing components were present in ethyl acetate extracts.

CONCLUSIONS

The study of the group composition of alcohol extracts from balsam poplar leaves (*Populus balsamifera* L.) collected on the territory of Krasnoyarsk (Russia) revealed various biologically active substances, such as megestrol acetate, γ-sitosterol, 22,2-dihydro-stigmasterol (sitosterol), *etc.* On the basis of these findings, dosage forms can be obtained, as well as components capable of suppressing the growth and development of microorganisms (3,7,11,15-tetramethyl-2-hexadecene-1-ol, diethyltoluamide, 4-methoxy-3-nitrobiphenyl, *etc.*).

It has been established that in the process of leaf development, the composition and quantitative content of some compounds change. This can be attributed to the physiology of the plant and the influence of external factors. For example, the maximum content of hydrocarbons of the alkane class, alcohols, individual compounds such as phytol (part of chlorophyll) is observed in July, after which the number of components decreases. After the leaf falls off, substances that have an insecticidal effect appear in its composition.

Thus, the study of alcohol-extractive substances of balsamic poplar leaves is promising for processing this type of waste in order to obtain biologically active drugs that can be used in various industries.

ACKNOWLEDGEMENTS

This work was carried out under the State Assignment issued by the Ministry of Education and Science of Russia for the project: “Technology and Equipment for the Plant Biomass Chemical Processing” by the Plant Material Deep Conversion Laboratory (Subject No. FEFE-2020-0016).

This work was performed using equipment of the Centre for Collective Use of the Krasnoyarsk Research Centre of the Siberian Branch of the Russian Academy of Sciences. We express gratitude to the staff of this centre for their assistance in our research.

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Article submitted: June 10, 2023; Peer review completed: August 5, 2023; Revised version received and accepted: November 5, 2023; Published: November 15, 2023.
DOI: 10.15376/biores.19.1.276-287