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Phytochemical analysis of extracts of wild plants growing in Yakutia for the content of basic groups of BAS

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ABSTRACTS

A process for extracting non-toxic, biologically active substances (BAS) from certain wild species of plants growing in Yakutia is described (Northeast of Russia). Using special qualitative reactions, phytochemical analysis of aqueous, aqueous-alcoholic, and alcoholic extracts of Pinus pumila (Pall) Regel, Thymus serpullum L., Artemisia yacutica Drob. for the content of basic groups of biologically active substances is performed.

Keywords: biologically active substances, plant extracts, thin layer chromatography, phytochemical analysis, flavonoids, coumarins, tannins, cinnamic acid, saponins, alkaloids, terpenoids, Yakutian wild plants.

ABBREVIATION*:*
BAS = Biologically Active Substances
WHO = World Health Organization
TLC = Thin Layer Chromatography
GOST = Set of technical standards in Russia
RMPM = raw medicinal-plant material
PTLC-F-A-UV 10x10 = *TLC plates with fluorescing in UV polyethylene terephthalate film 10x10cm*
TC = Technical Conditions, specifications
UV-254 = Ultraviolet spectrum with wavelength of 254 nm
C.P. = Chemically Pure
P.F.A.= Pure For Analysis
Deg = Reciprocal rotation tilt angle (degrees)
mBar = Pressure
R_f = *A measure of the speed of the motion of substances in relationship to each other during chromatography*
RPM = Rotational speed (revolutions per minute)
Vibro = Vibro rotation tilt angle (degrees)

INTRODUCTION

Herbal products are being used more and more in the World for the prevention and treatment of many diseases. Promising for these purposes are plant extracts whose activity is caused by the presence of certain groups of BAS [1-3].These BAS can have different compositions and belong to the flavonoids, glycosides, saponins, vitamins, phytohormones, etc. [1,4,5]. According to WHO, about 80% of people in the world today use herbal medicines for the prevention and treatment of various diseases. The advantages of herbal medicines include mild therapeutic effect, low toxicity, rare occurrence of side effects, and finally, affordability. The growing interest of the general population and healthcare workers in herbal medicine stimulates the expansion and renewal of the range of products by integrating plants used in folk medicine, encouraging the development of new herbal medicines having value for treatment and prevention, as well as perfume and cosmetics, and other plant-based products [6].

In the present work we have selected as objects of study several wild-grown plants showing medicinal promise growing on the territory of Yakutia. These all are used in the folk medicine of numerous other countries, as well as Yakutia [7,8]. Therefore, we have conducted phytochemical analysis of extracts of *Pinus pumila (*Pall*)* Regel,

Thymus serpullum L., *Artemisia yacutica* Drob. for their content of the basic groups of BAS - flavonoids, coumarins, tannins, cinnamic acid, saponins, alkaloids, terpenoids. To achive this goal we obtain aqueous, aqueous-alcoholic and alcoholic extracts of plants and using TLC we have performed qualitative estimation through data analysis and compared our data with published ones.

MATERIALS AND METHODS

Collection of plant materials

Fresh over-ground phytomass of *P. pumila*, *T. serpullum*, *A. yacutica* were collected in North-East (N 63°27′30.6″, E 142°54′′58.0″), Central (N 60˚31′09.0″, E 131˚26′26.7″) and Southern Yakutia (N 59°28′09″, E 119°17′37″) from 2011 to 2014 in accordance with generally accepted GOST [9-11] during the period from June through August. After post-processing, the phytomass was dried in an open way, in a well-ventilated place. The dried phytomass was milled [12] with a mortar and pestle and filtered through a 1-mm-diameter sieve and then stored under refrigeration in labeled kraft-paper packets.

Extraction of plant material

Extraction from the experimental plants was performed using 96% and 70% ethanol in a 1:10 ratio of raw material in two ways:

1) using Multi Rotator RS-60 (bioSan, Latvia) aqueous, aqueous-alcoholic and alcoholic extracts were obtained using the following settings: 40 rpm, 2° deg, 1° vibro, total extraction time 72 hrs. For aqueous extraction, 1 g of raw material pulverized to a particle size 1 mm in diameter was taken. 25 ml of deionized water (ratio 1:10) was used as an extractant. For aqueous-alcoholic and alcoholic extractions, 0.25 g of raw material pulverized to a particle size 1 mm in diameter were taken. As the extractant for alcoholic extraction, 12.5 ml of 70% ethanol were used; for aqueous-alcoholic extraction, 12.5 ml of deionized water and ethanol (1:10) were used.

2) using Universal Shaker LOIP LS 120 (Russia) aqueous-alcoholic extracts were obtained by following settings: shaking amplitude of 3 mm and total extraction time of 72 hrs. 2 g of raw material pulverized to a particle size of 1-mm diameter were used. The extractant was 200 ml of 96% ethanol and deionized water (1:10).

After extraction, filtration through a yellow ribbon paper filter d=15 cm (TC 2642-001-68085491-2011) was performed and the product dried in two ways:

1) using the lyophilizer Labconco 6l (USA) with a rotary vacuum pump and a drying chamber. Drying is done in special cans using the following settings: 1,250 mBar, at -84˚C. Duration of lyophilization of extracts depended on the volume (on average 3-4 hrs).

2) drying in a stream of air in the fume hood in evaporating dishes №3 and №4 with a diameter of 5.5 cm. The drying time was dependent on the volume of the extract (on average, 15-18 hrs).

Phytochemical analysis

As the basis for the phytochemical study of extracts of wild plants of Yakutia, thin layer chromatography was used. The process of the TLC experiment consists of the following steps: selecting and preparing the plates, applying samples on the plates, preparing separating mixtures, saturating the chromatographic chamber with the vapors of the separating mixture, executing the chromatogram, drying the plates, visualization of spots on the plates, performing qualitative reactions. Photochemical analysis done according to [4,13,14]

For TLC we used plates PTLC-F-A-UV TC 26-11-17-89* (10x10 cm) (stationary phase). The plates were washed in the main solvent of the separating mixture, and plates were dried in a low-temperature oven SNOL 67/350 (Latvia) for 1 h at 120˚C. To the prepared plates were applied the extracts which had been dried and dissolved in a small volume of methanol. The total amount of sample applied was 3 µl.

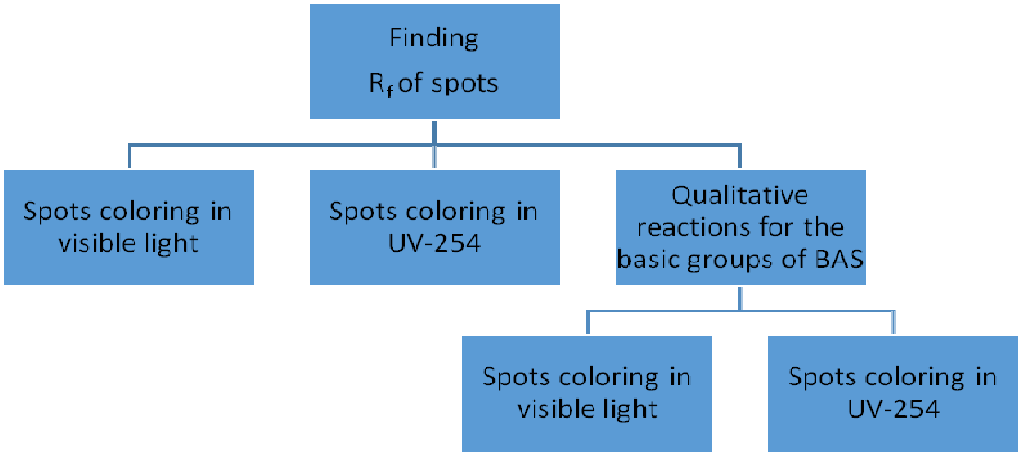
Chromatography was performed in the chambers, which had been pre-saturated for 30 min, within a solvent system in the ascending way, each chromatogram taking from 30 min to 2 h, depending on the type of solvents used for the separation and detection of the basic groups of BAS. The path length of the solvents was 7 cm. After drying, the plates were examined under visible light and UV lamp (254 nm). For TLC analysis, we used the following solvent systems, prepared several hours prior to chromatography, and stored in a refrigeration chamber at +4°C (Table 1). In this work we used c.p. and p.f.a. brands of solvents and reagents.

Table 1: Solvent systems for the detection of basic groups of BAS using TLC on silica gel plates (Sorbfil)

N	Group of BAS	Separating mixture (mobile phase)	Ratio
1	Flavonoids	1. Ethyl acetate : formic acid : glacial acetic acid : distilled water	100:11:11:22
		2. Chloroform : methanol	18:2
2	Coumarins	Ethyl acetate : benzene	1:2
3	Tannins	Benzene : chloroform	1:1
4	Cinnamic acid	Benzene : acetic acid : distilled water	4:1:2
5	Saponins	Chloroform : acetic acid : methanol : distilled water	6:2:1:1
6	Alkaloids	Methanol : ammonia (aq.)	17:3
7	Terpenoids	Benzene : ethyl acetate	1:1

TLC analysis was conducted in next way (Scheme 1).

Scheme 1: TLC analysis



RESULTS AND DISCUSSION

The healing quality of plants primarily depends on the content of BAS, which have diverse chemical natures and physiological effects [15]. The particular features of plant chemistry in the Yakutia region began to be studied at the beginning of the XX century [16-27]. Earlier studies of Yakutian flora discovered 36 families containing alkaloid species that belong to I, II and III groups of alkaloids [15]. Saponins have been found representing 136 species, 93 genera and 31 families [28-30]. 211 species representing 150 genera and 53 families of Yakutian flora have been examined for the content of tannins [16,31-33].

In the last five decades, due to the growing number of studies of the effects of synthetic and natural drugs on the human body, the greatest demand has been for drugs based on BAS of natural origin. Their distinctively important features are their harmlessness, their ability to be used constantly, and their wide range of biological effects [34,35]. Under more extreme growing conditionsplants synthesize more BAS [31,36-43], including flavonoids and flavonoid glycosides, alkaloids, saponins, vitamins, isoprenoids and their derivatives, organic acids, substances with antibiotic effect, regulators of replication speed, translation and DNA repair [38,41,44]. RMPMs with flavonoids in their compounds are widely used in clinical practice for a relatively long time [36].

The pharmacological properties of Artemisia implie the content of essential oils and sesquiterpene lactones [45-48]. The terpene fractions of essential oil of *A. yacutica* contain sabinene, a-pinene, 1.8-cineole, α-terpinene, bornylacetate, β-fapnezan, α- and β-humulene, δ-cadinene, intermedeol, bisabolol, selinan alcohol, and chamazulene [47].

The above-ground part of the *T. serpullum* contains essential oil, flavonoids, triterpene, thymine (saponic) (0.05%), ursolic, oleanolic, caffeic, chlorogenic and quinic acids, amarines, resin, tannins, and saponins [49]. The grass contains over 1% of essential oil whose main component is a crystalline thymol (up to 35%) and liquid carvacrol (up to 20%). The oil contains small amounts of terpenes: cymene, borneol, zingiberene, terpinene and terpeniol. In the grass were found triterpenes (ursolic and oleic acids) and flavonoids, tannins, amarines, and mineral salts. Mature seeds contain 33.6% of fatty oil. Acids in their composition: palmitic (2.6%), stearic (2.3%), oleic (11.4%) and linolenic (20.9%) [49,50].

The needles of *P. pumila* contain flavonoids: quercetin, kaempferol; higher fatty acids: tridecanoic, pentadecanoic, margarine, nonadecylic, heneicosane (n-henicosanoicacid, heneicosylicacid), tricosane, pentacosane; higher aliphatic alcohols: nonacosane-10 [51].

Yakutian plants are unique because the set of special climatic, hydrological, and biotic factors for wild plants have caused them to accumulate the full spectrum of BAS, which can make them suitable for the creation of herbal remedies for prevention and treatment, and for the purposes of biopharmacology.

Initially, we obtained extracts in two ways. In the first method, extracts were obtained using Multi Rotator RS-60 (bioSan). In the second method, a rocker-shaker was used. The first method requires a smaller amount of plant material and extractant (0.25 g of material and 12.5 ml of extractant). In the second method, 2 g of material, and 200 ml of extractant. In both cases of extraction there is a fairly equal extraction of BAS.

The separation of TLC can be optimized by attention to conditions when performing separation of samples on the plate [52]. For example, the composition of the mobile and stationary phases can be varied [51]. The amount of sample applied, and its correct application to the sorbent layer, plays an important role. Samples of the test substances were applied as 3 µl in dots not more than 3 mm in diameter. On the plate, the starting line was pre-marked at a distance of 1.5 cm from the bottom edge. The distance between the applied samples must be at least 1 cm. Then, the chromatography is performed. In the present work, the method of ascending chromatography was used. After the liquid is lifted to the specified height, the plate is removed and the front line is marked. Then the plate is dried in a fume hood under a stream of air. The values of R_f was recorded after each TLC.

Investigation of the phytochemical composition of Yakutian plants via qualitative reactions and TLC revealed the presence of the following groups of BAS: flavonoids, coumarins, tannins, cinnamic acid, saponins, alkaloids, and terpenoids.

For preliminary information about the structural features of the various compounds, we used chemical-analysis methods (qualitative reaction) on Sorbfil plates (PTLC-F-A-UV TC 26-11-17-89* 10x10). Photographs of the chromatograms obtained for detection of flavonoids (Fig. 1), coumarins (Fig. 2), cinnamic acids (Fig. 3), saponins (Fig. 4), alkaloids (Fig. 5), and terpenoids (Fig. 6) are shown. The results show a satisfactory separation of chromatographic zones of extracts on the chromatograms.

Phytochemical identification of different extracts (aqueous, aqueous-alcoholic and alcoholic) shows the presence of several compounds that were found by qualitative reactions [68]. Evaluation of the composition of extract of *T. serpullum* shows the presence of: glycosides, aglycones, flavanones, flavonols, chalcones, flavonolic glycosides, saponins, alkaloids, terpenoids, cinnamic acid, and catechin (Table 2).

Table 2. The presence of basic groups of BAS in various extracts of *T. serpullum*

Index	**Central Yakutia**			**Southern Yakutia**			**North-Eastern Yakutia**		
Color and flavor	The aqueous and aqueous-alcoholic extracts have a pleasant scent and a light-yellow color. Alcoholic extracts have a pleasant scent and green color.								
Type of extract	AE	AAE	AlcE	AE	AAE	AlcE	AE	AAE	AlcE
Flavonoids	+/-	+	+	+/-	+	+	+/-	+	+
Coumarins	+/-	+	+	+/-	+	+	+/-	+	+
Tannins	+/-	+	+	+/-	+	+	+/-	+	+
Cinnamic acids	+/-	+	+	+/-	+	+	+/-	+	+
Saponins	+/-	+	+	+/-	+	+	+/-	+	+
Alkaloids	+/-	+/-	+	+/-	+/-	+	+/-	+/-	+
Terpenoids	+/-	+	+	+/-	+	+	+/-	+	+

AE – aqueous extract; AAE – aqueous alcoholic extract; AlcE – alcoholic extract;
+ - the presence of compounds of BAS; +/- - the presence of BAS in small amounts

1. Reaction with Wilson's solution. After detection using Wilson's solution, no changes in chromatographic spots are observed. After drying in a stream of air, the color of spots does not change, and examination under UV-254 does not detect yellow-green fluorescence which would indicate the presence of 3- and 5-hydroxy flavones and 3- and 5-hydroxy flavonols.
2. Reaction with a 2% ethanolic solution of $AlCl_3$. After detection with this solution, no changes in chromatographic spots are observed. Spots after heating become orange-brown and begin emitting green light, indicating the presence of glycosides.
3. Reaction with a solution of NH_3. Chromatographic spots after detection with solution immediately become orange in color, which indicates the presence of flavones, flavonols and flavonones.

4. Reaction with vapors of NH_3. After detection with ammonia vapors for one hour, no changes in chromatographic spots are observed, no fluorescence detected, indicating the absence of anthocyanins.
5. Reaction with a 2% ethanolic solution of NaOH and KOH. During the detection with alkaline solutions, chromatographic spots become yellow and pink in color and emit a pink glow, indicating the presence of chalcones.
6. Reaction with a 1% alcohol solution of $AlCl_3$. Immediately after detection, chromatographic spots become blue in color and emit blue light, which indicates the presence of flavonoid glycosides.
7. Reaction with 1% solution of vanillin in concentrated HCl. After detection with solution, chromatographic spots do not become red-crimson, indicative of the absence of catechin, derivatives of phloroglucinol, and resorcinol.

From various extracts of *T. serpullum*, we selected those alcoholic extracts, which had shown positive results for the content of basic groups of BAS.

1. Reaction with 10% alcohol solution of NaOH and KOH. After detection, chromatographic spots take on a pink color with pinkish fluorescence, which indicates the presence of chalcones.
2. Reaction with a 1% alcohol solution of $AlCl_3$. After detection, chromatographic spots become yellow and emit yellow-green fluorescence, indicating the presence of flavonoid glycosides.
3. Reaction with 1% solution of vanillin in concentrated HCl. After detection, red-crimson coloring does not appear in the chromatographic spots, indicating the absence of catechins, derivatives of phloroglucinol, and resorcinol.
4. Reaction with a 1% $FeCl_3$. During detection, chromatographic spots on plates become yellow; when heated over an electric stove, some spots change color from yellow to intense brown. Green and brown glows appear under UV-254 indicates the presence of flavonoid glycosides.
5. Reaction with concentrated H_2SO_4. During detection with solution, spots become red-brown in color and emit brown fluorescence, indicative of the presence of saponin groups.
6. Reaction with 5 drops of concentrated H_2SO_4 in 10 ml of absolute methanol. After detection, chromatographic spots take on a brown color and brown fluorescence, which indicates the presence of alkaloid compounds.
7. Reaction with Wilson's solution. After the detection with solution, chromatographic spots have no changes.
8. Reaction with 10% solution of H_2SO_4. After detection with solution, chromatographic spots become reddish-brown in color and emit brown fluorescence, which indicates the presence of terpenoid compounds.
9. Reaction with vapors of J and H_2SO_4. After 15 min of being in iodine vapors, chromatographic spots on the plates turn brown, and when sprayed by H_2SO_4- turn red-brown with brown slaking, indicating the presence of saponin groups.

Evaluation of the composition of alcoholic extracts of *P. pumila* shows the presence of triterpenes, tannins, monoterpenes, catechins, cinnamic acids, flavonoid glycosides (Table 3).

Table 3. The presence of the basic groups of BAS in alcoholic extracts of *P. pumila*

Index	North-Eastern Yakutia
Color and flavor	Alcoholic extract have a pleasant scent and green color.
Flavonoids	+
Coumarins	+
Tannins	+
Cinnamic acids	+
Saponins	+
Alkaloids	+
Terpenoids	+

+ - the presence of compounds of BAS; +/- - the presence of BAS in small amounts

1. Reaction with vapors of NH_3. After exposure to ammonia vapors (8 min), chromatographic spots become green and brown in color with reddish-brown slaking, which indicates the presence of cinnamic acids.
2. Reaction with a 3% alcohol solution of $FeCl_3$. After detection with solution and heating, chromatographic spots turn purple, and after a while, they become blue with blue slaking under UV-254. New dots appear at R_f = 4,5 with gray and green fluorescence, which indicates the presence of flavonoid compounds.
3. Reaction with 10% alcohol solution of NaOH. After detection with solution, no changes in chromatographic spots are observed, after the heating spots turn yellow, but after a while, they become brown with reddish-brown fluorescence, which indicates the presence of terpenoid compounds.
4. Reaction with a 3% alcohol solution of $FeCl_3$. After the detection with solution and heating, chromatographic spots become gray-blue with gray slaking, which indicates the presence of terpenoid compounds.
5. Reaction with vapors of HCl. After exposure to vapors of HCl (8 min) and heating, new chromatographic spots appear at R_f = 0,5 with pale purple color and pale purple glow under UV-254, which indicates the presence of tannins.

Evaluation of the composition of alcoholic extracts of *A. yacutica* shows the presence of triterpenes, tannins, catechins, cinnamic acids, flavonoid glycosides (Table 4).
1. Reaction with vapors of NH_3. After exposure to ammonia vapors (8 min) chromatographic spots become green and brown in color with reddish-brown slaking, which indicates the presence of cinnamic acids.
2. Reaction with a 3% alcohol solution of $FeCl_3$. After detection with solution and heating, chromatographic spots become purple, and after a while, they turn blue with blue slaking, which indicates the presence of flavonoid compounds.
3. Reaction with 10% alcohol solution of NaOH. During the detection with solution, no changes on chromatographic spots are observed, but when heated, spots take on yellow color, which after a while become brown with reddish-brown fluorescence, which indicates the presence of terpenoid compounds.

Table 4. The presence of the basic groups of BAS in alcoholic extracts of *A. yacutica*

Index	Central Yakutia (The Botanical garden, Yakutsk)	Southern Yakutia	North-Eastern Yakutia
Color and flavor	Alcoholic extracts have a pleasant scent and a brown color.		
Flavonoids	+/-	+/-	+/-
Coumarins	+	+	+
Tannins	+	+	+
Cinnamic acid	+	+	+
Saponins	+	+	+
Alkaloids	+	+	+
Terpenoids	+	+	+

+ - the presence of compounds of BAS; +/- - the presence of BAS in small amounts

4. Reaction with a 3% alcohol solution of $FeCl_3$. After detection with solution and heating, chromatographic spots become gray-blue with gray slaking under UV-254, which indicates the presence of terpenoid compounds.
5. Reaction with vapors of HCl. After exposure to vapors of HCl (8 minutes) and heating, new purple chromatographic spots appear at R_f = 0,07, which indicates the presence of tannins.

For the detection of BAS we selected following solvent systems for TLC: for the detection of flavonoids: 1. Ethyl acetate : formic acid : glacial acetic acid : distilled water; 2. Chloroform : methanol; for the detection of saponins: Chloroform : acetic acid : methanol : distilled water; for the detection of alkaloids: Methanol : aqueous ammonia; for the detection of terpenoids: Benzene : ethyl acetate; for the detection of coumarins: Ethyl acetate : benzene; for tannins: Benzene : chloroform; for the detection of cinnamic acids: Benzene : acetic acid : distilled water. As a result, the optimal separation conditions of flavonoids found in the use of system "Chloroform : methanol" (18:2). Alcoholic extracts show better separation than aqueous extracts.

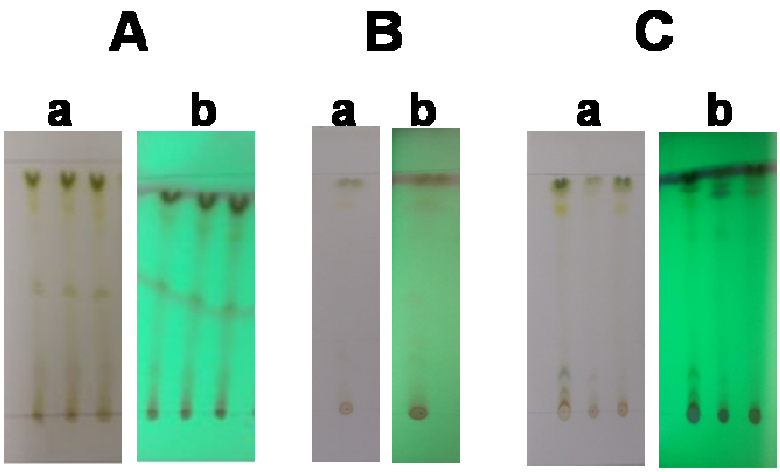


Fig. 1. The photographs of TLC plates for detection of flavonoids from alcoholic extract of plants. A) *T. serpullum*, B) *P. pumila,* C) *A. yacutica*. a) in a visible light; b) under UV-254

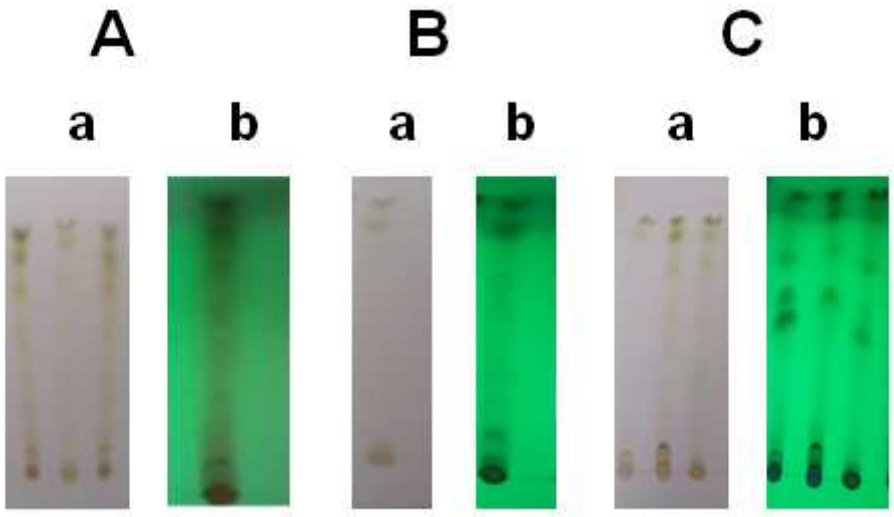


Fig. 2. The photographs of TLC plates for detection of coumarins from alcoholic extract of plants. A) *T. serpullum*, B) *P. pumila,* C) *A. yacutica*. a) in a visible light; b) under UV-254

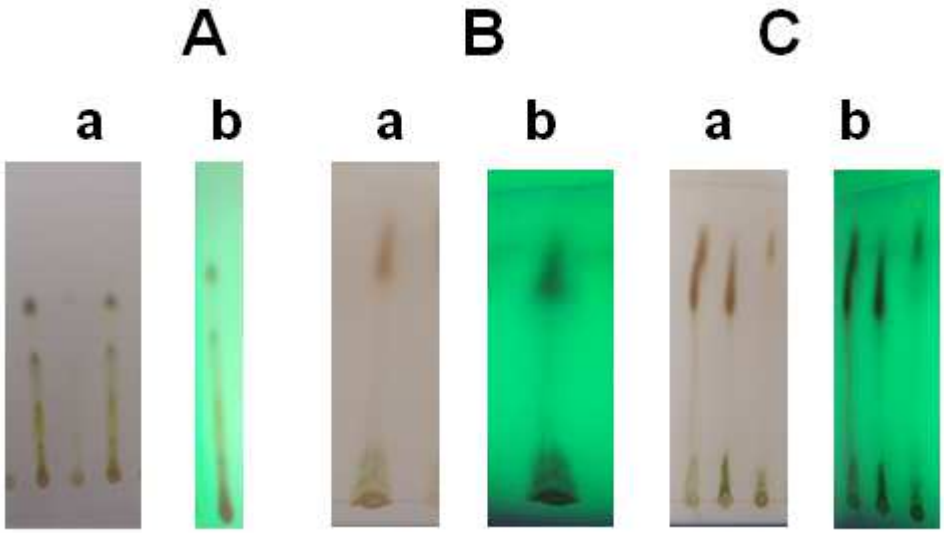


Fig. 3. The photographs of TLC plates for detection of cinnamic acids from alcoholic extract of plants. A) *T. serpullum*, B) *P. pumila,* C) *A. yacutica*. a) in a visible light; b) under UV-254

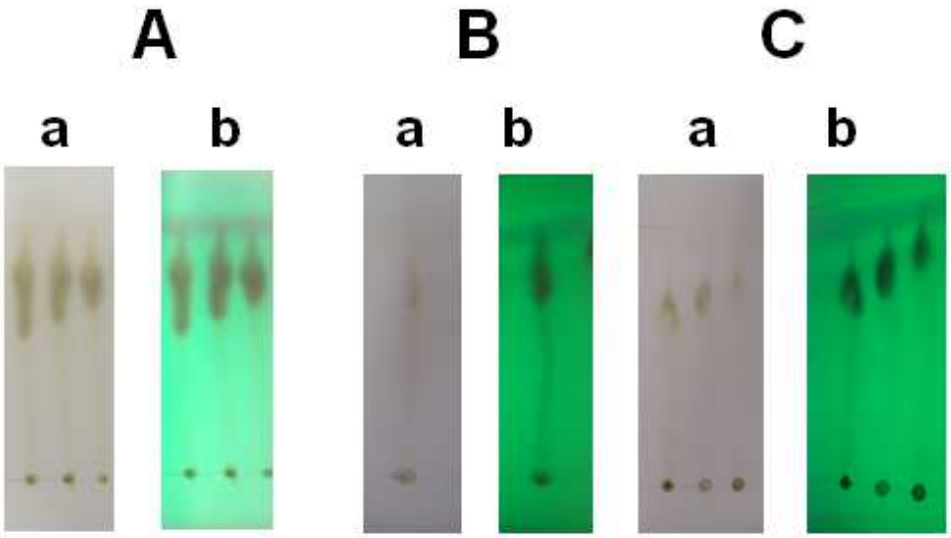


Fig. 4. The photographs of TLC plates for detection of saponins from alcoholic extract of plants. A) *T. serpullum*, B) *P. pumila,* C) *A. yacutica*. a) in a visible light; b) under UV-254

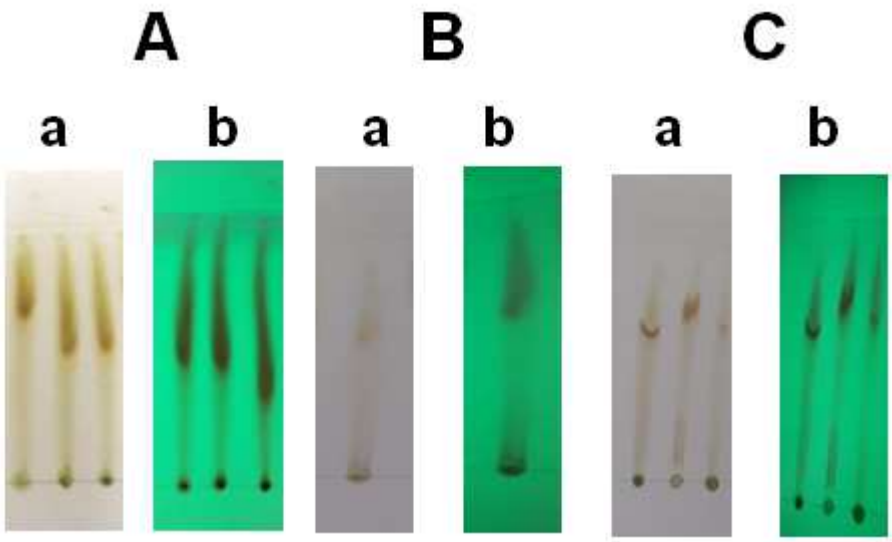


Fig. 5. The photographs of TLC plates for detection of alkaloids from alcoholic extract of plants. A) *T. serpullum*, B) *P. pumila,* C) *A. yacutica*. a) in a visible light; b) under UV-254

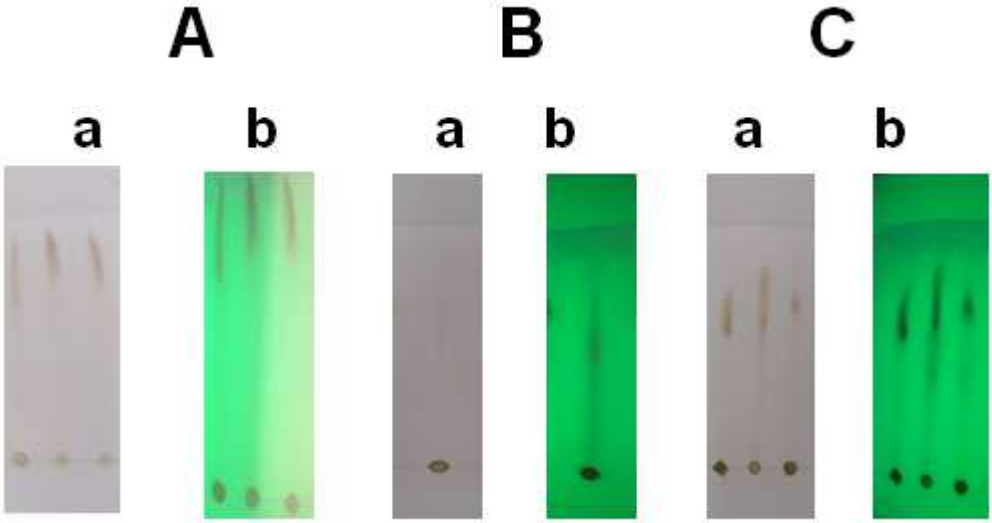


Fig. 6. The photographs of TLC plates for detection of terpenoids from alcoholic extract of plants. A) *T. serpullum*, B) *P. pumila,* C) *A. yacutica*. a) in a visible light; b) under UV-254

CONCLUSION

Despite the increased number of drugs produced synthetically, the herbal medicinal products are becoming more popular in medical practice. Up to 40% of all drugs used in medicine are obtained from plant materials [53]. For example, extractives of Siberian cedar foliage is a mixture of complex lipophilic compounds (fat-soluble vitamins, terpenoids, fatty and rosin acids, etc.) and hydrophilic components (phenolic compounds, water-soluble vitamins, etc.) used as drugs [54]. High performance chromatography detected caffeic acid derivatives and specific flavonoids in an alcoholic extract of *Artemisia balchanorum*. In an alcoholic extract of *thyme furrowed*, using the high performance chromatography, glycoside of luteolin and substances of flavonoid and flavonoid nature were found [55].

As a result of the qualitative analysis of extracts of the overground parts of *P. pumila*, *T. serpullum* and *A. yacutica* the presence of the basic groups of BAS was revealed, which determine the properties of the test plants. It has been found that flavonoids, saponins, alkaloids, terpenoids, coumarins, tannins, and cinnamon compounds are present to some extent indicating their pledges for further study.

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REFERENCES

[1]Y.V. Bataeva, *Natural Sciences*, **2011**, 3, 36.
[2]M.N. Zaprometov; Phenolic compounds, Moscow, Nauka, **1993**, 272.
[3]B. Carratu, *Ann. Ist. Super Sanita*, **2005**, 41, 1.
[4]M.A. Egorov, In: S.D. Varfolomeev, G.E. Zaikov, L.P. Krylova (Ed.), International Conference of Biochemistry and Biotechnology: Research and Development, 23 Jun. **2010**, New York (World Scientific, Russia 2010) 169.
[5]M. Sharshunova, V. Schwartz, I. Mikhailets; Thin layer chromatography in pharmacy and clinical biochemistry, Moscow, **1980**, 41, 607.
[6]T.S. Shestakova, PhD thesis, Perm State Pharmaceutical Academy (Perm, RF, **2007**).
[7]A.A. Makarov, N.A. Pryamkova, In: A.A. Makarov (Ed.), Materials to study medicinal flora in Yakutia (Yakutsk: Bk. Publishing House, **1977**) 8.
[8]D.N. Olennikov, N.K. Chirikova, Zh.M. Okhlopkova, & I.S. Zulfugarov, I. S, *Molecules*, **2013**, 18, 14105.
[9]GOST 6077-80. Vegetal medicine raw stuff. Packing, marking, transportation and storage.
[10]GOST 21769-84. Tree verdure. Specifications. Instead of GOST 21769-76; Added 01.01.1985; The latest publish date: 28.06.1984.
[11]State pharmacopoeia of USSR. 2nd ed., N.2. Moscow, **1990**, 400.
[12]GOST 24027.1-80. Methods for determination of authenticity, granary pest infestation, crushing and admixtures content. Moscow, **1982**. 25.
[13]J. Bekker; Chromatography. Instrumental Analysis: chromatography and capillary electrophoresis methods. Technosphera, Moscow, **2009,** 472.
[14] Chromatography. The practical application of the method: in two parts, E. Heftman (Ed.), Mir, Moscow, **1986**, 336.
[15]A.A. Makarov, *Plant resourses*, **1965**, 1, 3, 436-442.
[16]A.A. Makarov; Biologically active substances in plants of Yakutia. Publishing House, Yakutsk, **1979**.
[17]M.F. Gabyshev, A.V. Kazansky, Yakutian forage grass. Publishing House, Yakutsk, **1957,** 166.
[18]A.D. Egorov, Vitamin C and carotene in the vegetation of Yakutia. USSR Academy of Sciences Publisher, Moscow, **1954**, 248.
[19]A.D. Egorov, The chemical composition of forage plants of Yakutia. USSR Academy of Sciences Publisher, Moscow, **1960**, 336.
[20]A.D. Egorov (Ed.), Biochemical characteristics of plants of Yakutia (micronutrients and carbohydrates). Nauka, Moscow, **1964**, 210.
[21]A.D. Egorov, In: W.A. Tavrovsky (Ed.), Main results of biological study in the Yakutsk ASSR (Bk. Publishing House, Yakutsk, **1969**) 33.
[22]L.A. Znamenskaya, In: V.B. Kuvaev (Ed.), Vegetation data of Yakutia (VBO, Leningrad, **1961**) 253.
[23]P.N. Lashenkov, In: R.E. Regel (Ed), Study of the Bureau of Applied Botany (**1912**), 275.
[24]V.Y. Potapov, The carbohydrates and lignin in the forage grasses of Yakutia, Nauka, Moscow, **1967**, 174.
[25]V.Y. Potapov, A.D. Egorov, In: N.A. Avrorin (Ed.), Introduction of plants in Central Yakutia (Nauka, Moscow, Leningrad, **1965**) 182.
[26]N.I. Sharapov, The chemistry of plants and climate. Publishing House of the USSR Academy of Sciences, Moscow, Leningrad, **1954**, 208.
[27]N.I. Sharapov, Regularities of the chemistry of plants. Publishing House of the USSR Academy of Sciences, Moscow, Leningrad, **1962**, 130.
[28]V.P. Samarin, *Sc. rec. of Yakutisk Univ*., **1965**, 15, 15.
[29]V.P. Samarin, Poisonous plants of Yakutia, Yakutsk, **1966**, 194.
[30]V.P. Samarin, PhD thesis, Botany Institute of V.L. Komarov (Chelyabinsk, RF, **1970**).
[31]A.A. Makarov, In: A.V. Vlasov (Ed.), Problems of development of the productive forces of Yakutian ASSR, (Bk. Publishing House, Yakutsk, **1969)** 72.
[32]A.A. Makarov, Medicinal plants of Yakutia. Yakut Scientific Center of the USSR SB AS, Yakutsk, **1989**, 156.
[33]N.A. Pryamkova, In: V.N. Andreev (Ed.), Introduction of useful plants in Yakutia (NF SB AS USSR, Yakutsk, **1980**) 75.
[34]I.I. Brekhman, A.A. Grinevich, Biological resources of the eastern and southeast Asia and their usage. , FESC AS USSR, Vladivostok, **1978**, 143.
[35]N.N. Brehman, I.F. Nesterenko, Natural complexes of biologically active substances: sugar and human health. , Nauka, Leningrad, **1988**, 93.

[36]A.M. Goldovskiy, *Biology Bulletin Reviews*, **1941**, 14, 140.
[37]V.Y. Potapov, In: A.D. Egorov (Ed.), Biochemical characteristics of plants of Yakutia (micronutrients and carbohydrates). (Nauka, Moscow, **1964**) 161.
[38]V.V. Telyatev, Useful Plants of Central Siberia. East Siberian book publishing house, Irkutsk, **1987**, 400.
[39]V.G. Alekseev, A.A. Popov, B.M. Kershengolts, *Russian Journal of Plant Physiology*, **1983**, 30, 6, 1094.
[40]V.G. Alekseev, T.M. Shcherbakova, *Russian Journal of Plant Physiology*, **1983**, 30, 1, 73.
[41]V.G. Alekseev, Durability of plants in the North: Ecological and biochemical aspects. VO Nauka, Novosibirsk, **1994,** 152.
[42]G.V. Filippova, PhD thesis, Institute of Biological Problems of the permafrost zone (Ulan-Ude, RF, **2003)**.
[43]L.I. Brutko, T.D. Dargaeva, G.G. Nikolaeva, Quantitative analysis of herbal medicines. BF SB of AoS USSR, Ulan-Ude, **1985**, 150.
[44]V.L. Kretovich, Biochemistry of plants. Vysshaya Shkola, Moscow, **1986,** 503.
[45]D.D. Taran, In: N.R. Deryapa (Ed.), Problems of development of medicinal resources in Siberia and the Far East. (Novosibirsk, **1983)** 222.
[46]R.G. Kelsey, F. Shatizadex, *Photochemistry*, **1979**, 18, 10, 1591.
[47]S.L. Ivanov, *Works on applied botany, genetics and breeding*, **1924**, 13, 2, 483.
[48]K.F. Blinova, G.P. Yakovlev (Ed.), Botanical and pharmacognosical dictionary (Reference manual). Vysshaya Shkola, Moscow, **1990**, 246.
[49]A.D. Zorina, G.A. Fokina, A.L. Shavarda, A.M. Batjuk, *Plant resourses*, **2002**, 3, 60.
[50]I.N. Pustyrsky, V.N. Prokhorov, Universal Encyclopedia of Medicinal Plants. Moscow, **2000**.
[51]Plant Resources of Russia and Neighboring Countries: Part I - Family Lycopodiaceae - Ephedraceae, part II - Amendments to 1-7th volumes. St. Petersburg, **1996**, 571.
[53]Z.E. Mashchenko, PhD thesis, Perm State Pharmaceutical Academy (Perm, RF, **2004)**.
[54]T.V. Hurskainen, V.I. Terentyev, N.N. Skripova, A.A. Koroleva, A.V. Kuchin, *Chemistry of plant raw material*, **2014**, 1, 171.
[55]A.E. Paliy, V.N. Ezhov, I.N. Paliy, O.A. Grebennikova, G.V. Cornilev, In: Plugatar Yu.V. (Ed.), Collection of scientific works of GNBS, **2014**, 139, 107.