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INFLUENCE OF THE CONSTITUENT COMPOSITION ON ANTIMICROBIAL AND PERFUME PROPERTIES OF FIR ESSENTIAL OILS

The essential oils of 7 kinds of spruces by the method of steam distillation were obtained. The qualitative and quantitative composition of the received essential oils was studied by gas-liquid chromatography. Antimicrobial activity of essential oils on substances *Staphilococcus aureus* ATSS 6538 P, *Pseudomonas aeruginosa* ATSS 9027, *Bacillus subtilis* ATSS 6633, *Candida albicans* ATSS 885-653 was defined. Perfume tasting evaluation of the essential oils was made.

Introduction. Herbal preparations have unique properties that are not inherent to synthetically derived drugs in connection with the fact that plants contain all the substances necessary for human life and health. Preparations on the basis of vegetative raw materials represent a variety of complex substances, complexes of biologically active substances (BAS), which provide not only the therapeutic activity, but also preventive quality.

Approximately 25% of the prescription drugs contain at least one component is or once was obtained from plants [1].

In addition, herbal less than traditional antimicrobial agents, cause the formation of resistant strains. BAS plants can be an effective Supplement in the complex treatment of infectious diseases.

Nowadays about 320 species of medicinal plants are allowed for use in medical practice and it is a very small fraction of the total biodiversity of higher plants [2].

One of the reasons for the necessity of the development of plant biochemistry in the Republic of Belarus is searching for new promising herbal remedies.

Antimicrobial activity of various conifers is widely studied by scientists from Russia, Latvia, Turkey, Romania and Canada. The common pine (*Pinus sylvestris L.*), Norway spruce (*Picea abies (L) Karst*) and Siberian fir (*Abies sibirica L.*) are of the greatest interest [1, 3–4]. On the basis of essential oils of coniferous plants drugs for curative and preventive medicine, household chemical goods and agriculture have been obtained.

Spruce (*Picea*) – genus of the pine (Pinaceae) family – one of the main forest-forming and most cultivated species in the forest Fund of the Republic of Belarus. This kind consists of 36 species, of which 27 are widespread in Eurasia, 9 – in North America.

According to the statistical data of forest inventory 01.01.2012 its total stock was estimated at 184 million m^3. Spruce forests account for 9.8% of the forested area of the country, or 750 thousand ha [5].

In this regard, the object of our research was the essential oils of plants of the genus *Picea*: *Pungens*, *Glauca*, *Jezoensis*, *Abies*, *Obovata*, *Omorika* and *Mariana*.

The aim of the research was to study the biological activity and perfume values of essential oils representatives of the genus *Picea*, and to determine the opportunities for their use in medical practice and cosmetic industry.

To achieve the goals, the following tasks were set:

1. To study the chemical composition of the essential oils of wood greenery of fir-tree species growing under various conditions of technogenic load on the territory of the Republic of Belarus.

2. To investigate the biological activity of essential oils and to determine the minimal inhibitory concentration.

3. To have a tasting evaluation of perfume properties of essential oils.

4. To identify resource possibilities of using spruce foliage for obtaining biologically active substances.

Main part. Foliage was taken from a few trees that grow under the conditions of different anthropogenic load. The extraction of the essential oil was realized by hydrodistillation of the sample using a Clevenger's type device. Distillation was carried out by boiling 200 g of plant material freshly cut in 0.5 cm pieces approximately.

The analysis was done using a *Crystal 5000.1* gas chromatograph equipped with Rtx-1 capillary column (60 m * 0.25mm; film thickness 0.25 μm). Chromatographic conditions allow us to separate the individual components of the essential oil. 59 components of the essential oil spruce have been identified, which is 95–97 wt % of the total number of components.

Analytical conditions were: injector and detector temperatures: 250 and 190°C respectively.

The oven temperature is programmed from 70 to 150°C at 2°C/min, then isothermal at 150°C for 60 min; the carrier gas was 20 ml N_2/min. The constituents were identified by the combination of retention index data.

The main components of essential oils (content's greater than 1 wt %) are presented in table. 1.

Table 1

Constituent composition of essential oils of spruce, wt %

Compound	Spruce species					
	P. glauca		P. pungens		P. abies	
	1	2	3	4	5	6
cis-3-Hexanol	0.05	0.10	0.10	2.10	4.20	0.06
Trycyclene	1.01	1.27	1.57	1.28	1.29	1.61
α-Pinene	8.97	12.23	10.33	9.61	7.11	11.97
Camphene	18.00	25.50	16.58	15.84	18.60	19.12
β-Pinene	2.66	2.33	1.13	1.58	1.27	2.66
Myrcene	2.57	4.27	8.55	5.17	4.03	3.53
Δ^3-Carene	0.27	0.09	0.65	1.00	0.25	0.17
Limonene	6.15	2.35	12.52	11.90	8.76	14.73
1,8-Cineole	9.04	18.53	20.50	19.06	20.85	4.43
Terpinolene	1.38	1.11	0.62	0.95	0.40	0.65
Camphor	12.02	4.85	5.86	8.64	5.52	4.46
Citronellal	0.79	0.23	2.97	2.21	2.17	2.01
Borneol	2.70	0.72	1.46	2.11	3.76	5.99
Terpinen-4-ol	0.66	0.26	0.65	0.69	1.02	0.99
α-Terpineol	1.06	0.52	1.59	1.57	2.94	3.07
Bornylacetat	26.21	19.97	8.45	8.50	8.67	15.17
Total of identified	96.67	96.50	97.21	96.36	96.54	96.93

Component composition of the essential oils of various species of spruce remains constant, while the quantity of individual components is not only different in different species, but varies depending on growing conditions.

Glauca spruce is characterized by increased content of α-Pinene, Camphene, Myrcine and 1,8 Cineole if parts of Limonene, Camphor, Borneol and Bornylacetat are decreased.

The *pungens* spruce on the contrary has a decrease of α-Pinene, Camphene, Myrcene and 1,8 Cineole with increasing Borneol and Camphor.

P. abies contains the maximum number of cis-3-Hexenol ("the scent of fresh herbs") i.e. 4.20 wt %. The influence of cultivation conditions is the most noticeable with the increase in the content of Camphor and Bornylacetat.

The study of individual components did not find unequivocal dependence of the composition of the essential oil upon growing conditions and it caused the necessity to analyze the fractional composition of the essential oils samples (Fig.).

Research of changes of the fractional composition also does not have an explicit nature. The decrease of monoterpenoid compounds of *pungens* and *abies* spruce with a simultaneous increase of terpenoids due to catalytic oxidation reactions under the action of heavy metals corresponds to the data of work [6].

Solutions of essential oils in 10% ethanol were presented at the organoleptic evaluation to the Perfume Tasting Councilin in Moscow. Tasting was carried out on a test strip of filter paper.

The smell was evaluated in three stages:

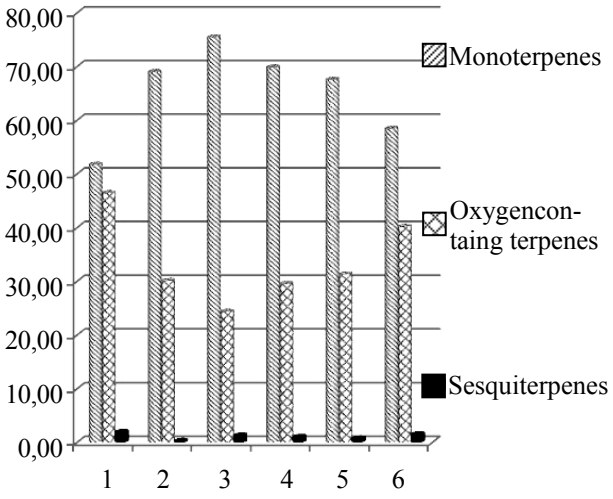
– for the first 2-5 minutes (top note);

– 20 minutes after the evaporation of the sample with a strip of filter paper (the heart)

– 45 minutes later after evaporation (base).

The Councilin pointed out the high quality of the presented samples: the absence of impurities, flavor and clarity.

The presented samples of essential oils of *mariana*, *glauca*, and *abies* spruce have received the highest marks (5), and have been recommended for the creation of perfumery compositions. Essential oils of *pungens* (4.5) and *omorica* spruce (4) were proposed to be used for creating cosmetics fragrances.



Fractional composition essential oils spruces

Essential oils of spruces were mainly characterized as wood-coniferous or grass-coniferous. However, it should be noted that *P.glauca* had a bright note of calamus root, *pungens* spruce – a flower note (lilac and Lily of the valley). *P.abies* was characterized by presence of citrus note.

Tests of samples of essential oils on the antimicrobial activity were carried out in accordance with the requirements of the State Pharmacopoeia of the Republic of Belarus with the use of test-cultures grampositive *Staphilococcus aureus* ATCC 6538-P, *Bacillus subtilis* ATCC 6633 and gramnegative bacteria *Pseudomonas aeruginosa* ATCC 9027, as well as fungi of *Candida albicans* ATCC 885-653.

All samples of essential oils possess antimicrobial activity against *Staphilococcus aureus* ATCC 6538-R. While diluting 1:10 essential oils of firs have activity against gram-negative bacteria *Pseudomonas aeruginosa* ATCC 9027. Essential oils of pungens and norway spruces also have activity against fungi.

To confirm the findings of the preliminary assessment of antimicrobial activity of spruce essential oils special research concerning the determination of antimicrobial activity was carried out in the laboratory of industrial microorganisms of the OB RUE "Belmedpreparaty".

Essential oils demonstrating the best results in the previous tests were selected as objects of research. Some of the specimens were obtained from spruce foliage growing with minimal anthropogenic load (1 – *P. glauca*, 3 – *P. pungens*, 5 – *P. abies*), and part – from foliage that grew in the city (2 – *P. glauca*, 4 – *P. pungens*, 6 – *P. abies*).

The test was carried out by serial dilution for the determination of the quantitative indicator characterizing the microbiological activity of antibiotic – the value of the minimum inhibitory concentration (MIC). To assess the sensitivity of microorganisms there used the nutrient medium – Hottinger broth containing 110–130 mg amino nitrogen. Bacterial suspension was prepared from the agar culture and added into nutrient medium in a quantity of 0.2 ml to the final concentration of 10^3–10^4 OE in 1 ml. Basic solutions of essential oils samples were prepared on the water purified with the addition of twin-80 in the amount of 1.5% to achieve a uniform suspending. Basic oil solutions were prepared at a concentration of 200,000 μg/ml for *Bacillus subtilis* ATCC 6633 and *Pseudomonas aeruginosa* ATCC 9027: 160,000 μg/ml for *Staphilococcus aureus* ATCC 6538-R.

Working solutions were prepared by the method of two-time serial dilution in Hottinger broth from the main solutions. The suspensions of microorganisms in the culture medium without the addition of essential oils were used for a control. Prepared inoculants were incubated for 48 h at a temperature of 35±1°C. The results were evaluated visually determining the presence or absence of growth in the medium containing different concentrations of tested samples of oils. The last test tube with growth retardation (clear broth) indicates the minimum inhibitory concentration in relation to this strain.

Results are presented in Table 2.

From the results it follows that the most sensitive to the action of fir oils are test-microorganisms of *Staphilococcus aureus* ATCC 6538-P, *Bacillus subtilis* ATCC 6633. Test-culture of *Pseudomonas aeruginos*a ATCC 9027 showed the lowest sensitivity to the action of the studied oils.

Best bacterial activity against Bacillus subtilis was shown by oil of *glauca* (1) and *pungens* (4) spruces, and against Staphilococcus aureus ATCC 6538-R – only the *Picea pungens* (4).

Resistance of gramnegative microorganisms, perhaps is due to the peculiarities of their structure, namely the presence of a double membrane, which prevents the penetration of oil.

According to [4], the main components that determine antimicrobial activity of European spruce, are α и β-Pinene, Δ^3-Carene, p-Cymene, Ocymene, Limonene, γ-Terpinene, Camphene, 1,8-Cineole, Linalool, Bornylacetat and Nerolidol. Total sum of these components is maximal in the *P.glauca* (2) and is 84.39 wt % from the sum of the identified components. For other essential oils, this is about 74.54 (1); 73.78 (3); 71.96 (4); 69.59 (5); 72.59 (6) wt %. The obtained results do not reveal clear regularities and require further research.

Table 2

The experimental values of the minimum overwhelming concentration of essential oil spruces

Test-microorganisms	MIC, μg/ml					
	1	2	3	4	5	6
Pseudomonas aeruginosa ATCC 9027	100,000	100,000	–	100,000	100,000	–
Bacillus subtilis ATCC 6633	781.25	6,250	50,000	781.25	3,125	12,500
Staphilococcus aureus ATCC 6538-P	10,000	10,000	10,000	625	5,000	5,000

Note. "–" denotes the absence of stunting strain of microorganisms in vitro.

Conclusion. Essential oils of fir trees growing under natural conditions and under conditions of technogenic pollution have been obtained.

The terms of chromatographic analysis of spruce oils, providing maximum separation of the components with the content of 0.01 wt % have been selected.

Terpenic composition of essential oils of the seven species of fir-trees was studied. Interspecies dependence in the change of the fractional composition was not found. It is established that the greatest content of monoterpenic faction (75.42%) was observed in the *P. pungens*, growing in natural conditions. Essential oil of clean *glauca* is characterized by the highest content of terpenoids (46.40%). *P. mariana, glauca and abies* have the biggest value for perfumery and cosmetic industry possibly due to the high content of limonene, linalool and citronellal in its composition (from 9% to 20%), which providing a citrus note in the given oils.

Essential oils have different antimicrobial activity depending on the type of spruce. The essential oils of firs demonstrate the greatest activity against gram-positive bacteria.

Minimum inhibitory concentration of essential oil of pungens spruce against *Staphilococcus aureus* ATCC 6538-R is 625 μg/ml, which allows us to consider it as a raw material for production of medicinal preparations against this strain of microorganisms.

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