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Waste management of dark coniferous trees debarking for producing antibacterial preparations

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Abstract. The article presents the results of studying the antibacterial activity of fir tree and Siberian spruce bark water extracts. As test objects, pathogenic (3-4 hazard class) and saprotrophic bacteria were used. It has been shown that the studied aqueous extracts of dark coniferous bark do not exhibit biocide activity against the conditionally pathogenic bacterium *Escherichia coli*. However, all the other studied bacteria are sensitive to the extracts analyzed both on day 3 and day 13. At the same time, the aqueous extract of the Siberian fir bark has a higher biocide activity against conditionally pathogenic and saprotrophic bacteria in comparison with the aqueous extract of the Siberian spruce bark. The antibacterial activity of aqueous extracts of the studied plants bark is determined by their chemical composition, primarily the presence of substances of a phenolic nature. It has been shown that the studied extracts mainly contain compounds of quercetin, dihydroquercetin and kaempferol.

1. Introduction

According to experts, about half of the world's coniferous trees stocks are concentrated in the Russian Federation. To use only a tree stem is a standard practice in timber harvesting. Enterprises of the timber and wood-products industry and the chemical-timber complex receive wood mainly unpeeled. As a result, about 30m m³ of bark in the form of debarking waste is formed at these enterprises annually [7].

Virtually all of the bark is exported to bark dumps or burned. At the same time, the resulting products of combustion adversely affect the environment, and the accumulating deposits of waste litter the area, pollute water bodies and pose a certain fire hazard [2]. Disposal of these wastes, their involvement in industrial processing is a highly relevant economic and environmental task.

It is known that the bark of coniferous trees in comparison with deciduous species is richer in terpenes, polyphenols, lignin, and other compounds that are toxic to microorganisms [9]. In this regard, one of the possible directions for the use of dark-coniferous bark can be obtaining antiseptics for processing specialized wood products: children's furniture, trays for bakery products, kitchen utensils, etc.

Our earlier studies of the bactericidal activity of aqueous extracts of Siberian larch bark [3] showed that the latter are active against conditionally pathogenic and saprotrophic bacteria. Polyamide fiber dyed with the studied extracts exhibits bactericidal activity against the culture of *Bacillus amyloliquefaciens*. Moreover, this property is preserved after five washes. This circumstance allows us to recommend polyamide yarn dyed from the bark of Siberian larch as a raw material for the manufacture of hosiery with antibacterial properties [4].



In this study, the focus was on the bactericidal activity of aqueous extracts of Siberian fir bark (*Abies sibirica*) and Siberian spruce (*Picea obovata*).

2. Experimental part

Spruce and fir bark of fresh debarking of wood processing enterprises of the Angara-Yenisei region was used as a raw material. The bark was dried at a temperature of 105°C for 5-6 hours, ground and a fraction of 5-10 mm was selected for testing. The aqueous extraction was carried out for one hour in a flask under reflux at a boiling point of water and a water ratio of 20. After cooling, the resulting extract was filtered through a filter paper (white ribbon).

As test objects, museum cultures of sanitary indicative potentially pathogenic (3-4 hazard class) bacteria were used: *Klebsiella pneumonia* T 904, *Escherichia coli* ATCC 39/21141, *Staphylococcus aureus* ATCC 25922, *Proteus vulgaris* and saprotrophic bacterium *Micrococcus luteum*, obtained from the All-Russian collection of microorganisms and the FSBI SCEEMP (Scientific Centre for Expert Evaluation of Medicinal Products). Potentially pathogenic bacteria, to varying degrees, are capable of causing septic complications and are characterized by increased resistance to many disinfectants and antibiotics. These bacteria are sanitary indicative and are often found with complications of various diseases in clinics. Saprotrophic bacteria have antagonistic activity to many microorganisms (for example, to phytopathogenic). They are often found in soil, water, on food, where they can cause produce rotting by developing in large quantities.

Bacteria were grown on a dense nutrient medium - meat-peptone agar (MPA). For this, the nutrient medium was seeded with a lawn test-culture. Then a sterile disk of filter paper was placed in the center of the Petri dish, and 200 mcl of the water extract under investigation was sterilely applied to the surface of the filter paper. The extract gradually diffused into the agar, creating a dark brown colored area around the disk. After that, Petri dishes were incubated in a thermostat at 27°C. Accounting for the effect of the studied aqueous extracts of dark-coniferous bark on the growth and development of bacteria was carried out on the 3rd and 13th day. As a control, 200 mcl of sterile water was applied to the filter disc.

To study the chemical composition of aqueous extracts of Siberian fir and Siberian spruce bark, a method of thin-layer chromatography on Silufol plates (silufol UV 254) was used - polymer plates measuring 10 x 15 cm with a layer of silica gel containing an inert inorganic luminescent indicator.

Due to the fact that the main substances of the studied biomass with bactericidal activity, by their nature, are flavanoids (as evidenced by cyaniding test, boron-lemon reaction and reaction with ammonia solutions), it is optimal to use a mixture of solvents to separate them: n-butanol - acetic acid - water in the ratio of 4:1:5.

In order to remove ballast substances of a polycarbohydrate nature, the aqueous extract was pretreated with 1.5-3.0 ml of a 2.0% solution of egg protein. Excess albumin was removed from the aqueous extract by heating to a temperature of 85-90°C.

The separation of the flavonoids of the Siberian fir and Siberian spruce bark in a thin layer was carried out by the usual ascending method. For this, a drop of the prepared aqueous extract was applied with a capillary on the starting line on the plate. The plate was placed in a chamber for chromatography with a layer of a mixture of solvents. The lower end of the plate was immersed in the eluent no more than 5 mm. Pre-saturation of the chamber with eluent vapors greatly affects its speed of movement. In this regard, the chromatography was carried out in a closed chamber. Processing time is 60 minutes. Then the chromatogram was dried in air and the coefficient of movement R_f was calculated for each spot.

Identification of individual substances was carried out by comparing the calculated value of the coefficient of motion with tabular data.

3. Discussion of the results

Water-soluble substances were extracted from the bark of the studied dark coniferous plants: Siberian fir (*Abies sibirica*), Siberian spruce (*Picea obovata*) and their bactericidal activity was studied. The research results are presented in the table 1.

Table 1. Effect of an aqueous extract of Siberian fir and Siberian spruce bark on potentially pathogenic and saprotrophic bacteria.

The researched preparation	K. pneumonia		P. vulgaris		S. aureus		E. coli		M. luteum	
	72 h	312 h	72 h	312 h	72 h	312 h	72 h	312 h	72 h	312 h
Aqueous extract of Siberian fir bark	-	-	-	-	-	-	+	+	-	-
Aqueous extract of Siberian spruce bark	-	+	-	+	-	+	+	++	-	-

^a «+» – presence of test cultures growth
 «-» – lack of test cultures growth

In the course of the microbiological studies, it was established that *Escherichia coli* is not sensitive to the studied aqueous extracts. Its growth has spread all over the agar; no growth zones around the filter discs were detected. However, all other test cultures were sensitive to the studied extracts.

The most active aqueous extract of the Siberian fir bark restrained the growth of bacteria for 3-5 days, but also on the 13th day a similar effect was observed. In addition, in the case of *Proteus vulgaris*, the no-growth zone increased by 13 days (figure 1). More sensitive bacteria to the action of this extract were *Klebsiella pneumonia*. As can be seen from the diagram presented in figure 1, it is in the case of this bacterium that the largest zone of no-growth is recorded around the filter disc.

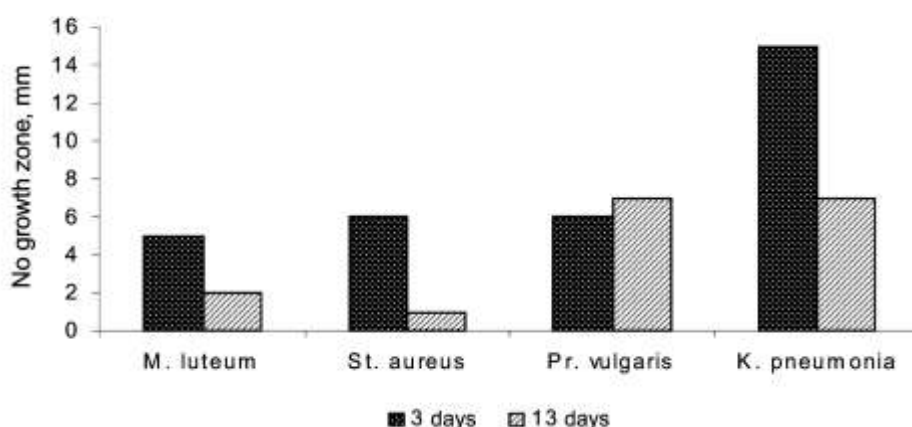


Figure 1. Effect of an aqueous extract of Siberian fir bark on test bacteria.

Aqueous extract of Siberian spruce bark has less bactericidal activity than an aqueous extract of Siberian fir bark. In the course of the observations it was identified that its maximum activity falls on 1-3 days. Further, the antibacterial effect of the extract of *Picea obovata* is declining and for 13 days the presence of growth of all tested potentially pathogenic cultures is recorded (figure 2).

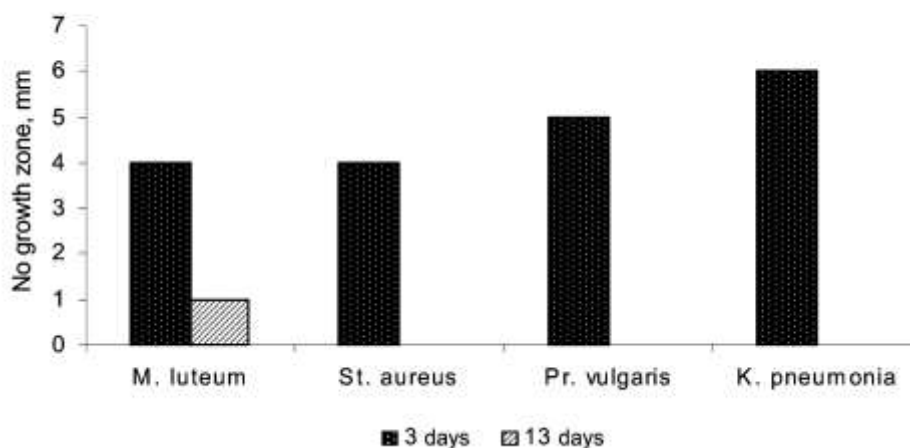


Figure 2. Effect of an aqueous extract of Siberian spruce bark on test bacteria.

The chemical composition of the Siberian fir and Siberian spruce bark has been studied quite well. So, Ostroukhova L A with co-authors found that water-soluble substances in the bark of Siberian fir contains 10% of the absolutely dry sample, and in the case of the Siberian spruce bark - almost 7%. At the same time, the share of phenolic compounds in the total content of extractive substances is 97.8 and 42.4%, respectively [5].

By the research of Fyodorova T E and Babkina V A [8], it was shown that phenolic compounds of Siberian spruce bark are represented by 17-18% flavonoids, 28-30% glycosides, and about 15% are oligo- and polyphenolic compounds.

Data on the study of phenolic extractives of the Siberian fir bark is very limited. So, the study of Gromova A S [1] indicates that the latter are mainly represented by phenolic acids and flavanoids.

The results of the current study of the chemical composition of phenolic compounds of the Siberian fir and Siberian spruce bark showed that mainly quercetin ($R_f = 0.77$), dihydroquercetin ($R_f = 0.81$) and kempferol ($R_f = 0.90$) are present in aqueous extracts, which is consistent with data obtained by other researchers [5].

The release of flavonoids in a pure form is possible when processing solutions with lead acetate. As a result, it was found that flavanoids are present in aqueous extracts of the Siberian fir and Siberian spruce bark in pure form and in the form of esters. This is confirmed by the extraction of components with polar and non-polar solvents. Moreover, the ratio of flavanoids and their esters in the studied biomass is approximately 1:1, as evidenced by almost the same optical density of the corresponding solutions.

Available in the literature data on the high biological activity of flavonoids [6, 9, 10] enables to predict the relevance of phenolic compounds from the waste of dark coniferous trees in the manufacture of a wide range of antiseptic, pharmacological and other preparations. The solid residue of the bark after extraction can also be used as mulch to improve the soil structure, the basis for the production of sorbents, fillers, etc.

4. Conclusions

The bactericidal activity of aqueous extracts of the Siberian fir bark (*Abies sibirica*) and Siberian spruce (*Picea obovata*) has been studied.

It has been found that freshly prepared aqueous extract of the Siberian fir bark has a higher biocide activity against potentially pathogenic and saprotrophic bacteria in comparison with the aqueous extract of the Siberian spruce bark.

It has been shown that an aqueous extract of Siberian spruce bark retains antibacterial activity for 3 days, and a similar extract of Siberian fir bark - for at least 13 days.

The studied aqueous extracts of dark coniferous bark do not show biocide activity against the potentially pathogenic bacterium *Escherichia coli*.

The antibacterial activity of aqueous extracts of Siberian fir and Siberian spruce bark is determined by their chemical composition, primarily the presence of substances of a phenolic nature. It has been shown that the studied extracts mainly contain compounds of quercetin, dihydroquercetin and kaempferol. Moreover, the ratio of flavanoids and their esters is approximately 1:1.

Thus, the chemical composition and bactericidal activity of aqueous extracts of Siberian fir and Siberian spruce bark indicate the presence of high potential in the debarking of dark coniferous trees for the pharmaceutical, cosmetic, textile, and agricultural industries.

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