ANTISEPTIC BANDAGING MATERIALS BASED ON SPHAGNUM

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*The research focuses on preclinical studies of new antiseptic surgical bandaging material with a high absorption capacity based on plant raw material — sphagnum. In folk medicine, sphagnum is known as good antiseptic material. Partisans used it particularly actively in marshy forests of Belarus saving the wounded from death during the Great Patriotic War. Despite the fame in folk medicine, until now there is no industrial production of bandaging material on its basis. The possibility of practical solution of the issue of import substitution of bandaging materials with the help of this study confirms its significance. Besides, in literature, there is virtually no information on physical and chemical characteristics of sphagnum and reasons for its antiseptic properties. In this study, the composition of compounds giving antiseptic properties to sphagnum was investigated with the use of modern instrumental methods. The content of trace elements in the sphagnum composition was determined. A prototype of bandaging material was made, its ability to show antiseptic properties towards trace elements and fungi of *Staphylococcus aureus*, *Actinomyces* sp, *Candida* spp., *E.Coli* and *Bacillus cereus* was assessed. The conducted studies showed the fundamental possibility of creating new surgical bandaging materials on the basis of sphagnum with better performance characteristics in comparison with cotton-gauze bandaging materials.*

Key words: antiseptics, bandaging material, trace elements, analysis.

Post-operative daily wound dressing causes unpleasant procedures related to irritation and injury of regenerating tissues resulting in bandage becoming a painful procedure for a patient. In this regard, it is necessary to search for bandaging materials having antiseptic properties for a long time and capable of absorbing wound effluents in large quantities. With the presence of such bandaging materials, there is no need for their frequent daily replacement.

The aim of this study is to check the possibility of creating antiseptic bandaging materials of high absorption capacity based on domestic raw materials. Sphagnum plant material was used as the main substance. We studied chemical compositions of sphagnum extracts using organic solvents, mass-spectrometric and IR-spectrometric methods. With that, the presence of various classes of compounds having antiseptic properties was found. Bandaging materials were made and tested for antiseptic activity towards various microorganisms. In addition, it was found that with long (over 10 years) storage the bandaging material does not lose its antiseptic properties.

Test samples are made by needle punching method with the punch frequency of 100–120 punctures per 1 cm² in the form of three-layer cloth, the upper and lower layers of which consist of synthetic fibre, and the middle layer contains sphagnum dried and crushed to sizes of 50–150 μm. The method of determining the sensitivity of microorganisms to antimicrobial compounds by the degree of their growth delay was used as a method for assessing antiseptic properties of experimental bandaging materials. The relative content of trace elements in sphagnum was determined by spectral method. In this respect, 23

trace elements were found in sphagnum, including phosphorus, silicium, aluminium, which stimulate growth and development of bone, connective and epithelium tissues, as well as facilitate recovery and regeneration processes.

Materials and methods

In order to find out the presence of organic substances giving antiseptic activity to sphagnum, it was extracted with toluene (C₆H₅-CH₃), carbon tetrachloride (CCl₄), water (H₂O), acetone (C₃H₆O), and ethyl alcohol (C₂H₆O), and studies of the composition of these extracts were carried out. Extraction was carried out on the Soxhlet extractor at a temperature of 80–100°C for 3 hours. The ratio of sphagnum to solvents amounted to 1:20 g/g. The resulting extract was filtered through a paper filter and used for testing.

The identification of substances extracted by the above solvents was carried out using mass spectroscopy in combination with gas ion chromatography on the MAESTRO GH 7820 chromatograph [1, 2]. IR-spectrums were fixed on the JASCO FT/IR-6800 IR-Fourier spectrometer [3].

The sphagnum extract was obtained on the Soxhlet extractor. The ratio of sphagnum : solvent = 1:20 g/g. The extraction was conducted while boiling of solvents for 3 hours. The solution was filtered through a paper filter and studied with mass spectroscopy and IR-spectroscopy for the presence of organic substances in the studied sphagnum extracts.

Then, studies on the antimicrobial effect of pure sphagnum and experimental bandaging material were carried out. For this purpose, we used methods based on the ability of antibiotics to diffuse in the microbial environment and to delay growth or even kill microorganisms in the

antiseptic diffusion zone [4]. The microorganisms of *Staphylococcus aureus*, *Actinomyces sp.*, *Candida spp.*, *E.Coli*, and *Bacillus cereus* were used as test objects.

The studies were carried out on samples made by needle punching method, they consist of three layers: the upper and lower layer made of synthetic fibre, the middle layer made of shredded sphagnum 4-5 mm thick [5].

To determine the effect of sphagnum on the selected test object, the following methods were used: streaking method, disk diffusion test, and volume displacement method. Data on growth suppression in the sphagnum presence were determined in two days at a temperature of 37°C.

The content of trace elements in sphagnum was identified through dividing the stems into three sections, as shown in Figure 1.

Figure 1. Division of the sphagnum stem for the studies on trace elements.

The presence of trace elements in sphagnum and their relative content were determined on the SPM-35 X-ray multichannel spectrophotometer.

For spectral studies, samples in the amount of 10 g were gradually heated for 30 minutes to a temperature of 1100°C and calcinated at this temperature for 30 minutes in a muffle furnace. The resulting ash was examined for the presence of trace elements.

Results and discussion

The fine interpretation of mass spectrograms of sphagnum extracts in toluene, carbon tetrachloride, ethyl alcohol, and water showed the presence of such substances as ethyl benzene, benzyl carbinol, acetophenone, phenylmethanol, benzene, phenol, acetic acid, styrene, etc. in tangible quantities. These substances contain aromatic nuclei or alcohol hydroxyl in the molecular structure, which give sphagnum antiseptic properties (Table 1). We did not consider in medical practice the presence of small amounts of hydrocarbons not being antiseptics.

It can be seen that sphagnum extracts contain compounds with aromatic rings being good antiseptics in medical practice.

For additional clarification of the chemical structure of macromolecular objects of sphagnum extracts, their IR-spectroscopy [3] was carried out on the FT-801 spectrometer. The frequency interpretation is presented in Table 2.

Table 1

Results of mass spectroscopy of sphagnum extracts in solvents

Frequency	Content of substances in solvents, %				Component
	$C_6H_5CH_3$	Cl_4	H_2O	C_2H_5OH	
8.593	6.06	–	–	–	Ethyl benzene: C_8H_{10}
13.282	0.30	–	–	–	Benzene, (1-methylbutyl): $C_{11}H_{16}$
617.904	0.46	–	–	–	Acetophenone: C_8H_8O
18.392	5.32	–	–	–	Phenylmethanol: $C_8H_{10}O$
9.708	–	0.29	–	–	Ethyl benzene: C_8H_{10}
19.616	–	0.26	–	–	Phenylmethanol: $C_8H_{10}O$
2.001	–	–	0.03	–	Phenol: C_6H_6O
2.208	–	–	0.02	–	Glycolaldehyde dimer: $C_4H_8O_4$
2.302	–	–	0.06	–	Acetic acid: $C_2H_4O_2$
2.843	–	–	0.02	–	Acetol: $C_3H_6O_2$
1.941	–	–	–	7.49	1-propanol: C_3H_8O
10.126	–	–	–	7.04	Styrene: C_8H_8

Table 2

Frequencies of IR-spectrum of sphagnum extract in solvents

Frequency ν , cm^{-1}	Compound classes in toluene
1740.94	Limit aliphatic $-\text{CH}_2-\text{CHO}$, cyclopentanone, alpha-halogen acids $\text{CH}-\text{COOH}$, carboxylic acid anhydrides $-\text{CO}-\text{O}-\text{CO}-$ $\nu_{\text{C=O}}$
1581.26	Polyenes, primary amines NH_2 , pyridines and quinolines, pyrimidines and purines
1516.20	Pyrimidines and purines, thiophenes
1463.65	Nitrosamines $\text{R}_2\text{N}-\text{N}=\text{O}$, boron compounds BCH_3 B-Ar,
1160.27	Propionates, higher esters, phosphoric acid esters $(\text{RO})^3\text{P}^+ + \text{O}^-$
1106.86	Tertiary alcohols, aromatic acid esters, thiocarbonyl compound derivatives, benzene compounds
1023.24	Aromatic and vinylic $=\text{C}-\text{O}-\text{C}-$, aliphatic amines, sulfur-containing functional groups
722.78	Alkanes, sulfur-containing functional groups
	Compound classes in CCl_4
3306.80	Primary amines $\text{R}-\text{NH}_2$, secondary amines $\text{R}_2\text{N}-\text{H}$, amine salts NH_4^+ , boron compounds BOH B-H-B
2922.63	Sulfur-containing functional groups $\text{R}-\text{SO}-\text{OH}$
2643.25	Boron compounds, boranes $\text{R}-\text{BH}_2$
1740.94	Limit aliphatic $-\text{CH}_2-\text{CHO}$, cyclopentanone, alpha-halogen acids $\text{CH}-\text{COOH}$, carboxylic acid anhydrides $-\text{CO}-\text{O}-\text{CO}-$ $\nu_{\text{C=O}}$
1581.26	Polyenes, primary amines NH_2 , pyridines and quinolines, pyrimidines and purines
1516.20	Pyrimidines and purines, thiophenes
1463.65	Nitrosamines $\text{R}_2\text{N}-\text{N}=\text{O}$, boron compounds BCH_3 B-Ar,
1160.27	Propionates, higher esters, phosphoric acid esters $(\text{RO})^3\text{P}^+ + \text{O}^-$
1106.86	Tertiary alcohols, aromatic acid esters, organic compound halogen derivatives, thiocarbonyl compound derivatives, benzene compounds, organophosphorous compounds
1023.24	Aromatic and vinylic $=\text{C}-\text{O}-\text{C}-$, aliphatic amines, sulfur-containing functional groups
722.78	Alkanes, sulfur-containing functional groups

The obtained data indicated that sphagnum contains in its composition a wide range of various compound classes showing antiseptic properties towards various groups of microorganisms. These are organochlorine and aromatic compounds, organophosphorous, primary and secondary aminocompounds, various alcohols. Data of IR-spectrums of sphagnum extracts give a wider range of qualitative composition of substances compared to mass spectroscopy. The results of studies show the possibility of using sphagnum as an antiseptic component of bandaging materials.

Using sphagnum finely crushed to the sizes of 50-150 μm , we made test samples of bandaging material and tested for antiseptic activity towards

various microorganisms. The effect of test bandaging materials on various microorganisms was examined through different methods: streaking method, disk diffusion test, and volume displacement method [4]. The results of characteristics of bandaging material test samples are presented in Table 3.

The made material is a composite of synthetic fibre for forming the shape and of sphagnum as the main antiseptic component with high absorption capacity (Table 3, exp. 1, 2). With the increase in the content of sphagnum, its absorption capacity naturally increases (exp. 3–8). The made bandaging material was further tested for antiseptic activity, the results are presented in Table 4.

Table 3

Composition and physical and chemical characteristics of samples

No. of experiments	Composition, % of mass		Water absorbing capacity, g/g	Density, g/cm ³	Ash content, %
	synthetic fibre	sphagnum			
1	-	100	16–18	1400	3.14
2	100	-	3–4	1800	4.1
3	35	65	17.1	1800	-
4	45	55	14.5	1840	-
5	50	50	14.0	1810	-
6	70	30	11.0	1740	-
7	85	15	5.2	1700	-
8	90	10	4.7	1600	-

Table 4

The assessment of antiseptic activity of bandaging material samples towards various microorganisms

No. of experiments	Composition of samples, % of mass		Microorganism growth suppression, mm								
	synthetic fibre	sphagnum	Staphylococcus aureus			Candida spp.			Astinomyces sp.		
			streaking method	disk diffusion test	volume displacement method	streaking method	disk diffusion test	volume displacement method	streaking method	disk diffusion test	volume displacement method
1	35	65	45.0	63.9	16.7	13.1	31.1	14.6	10.4	83.1	86.6
2	45	55	45.0	58.9	13.7	10.8	27.5	14.1	9.8	82.8	85.5
3	50	50	45.9	56.7	15.2	9.5	25.5	13.5	9.4	80.1	85.1
4	70	30	43.2	46.8	6.2	4.7	18	12.7	4.7	76.1	83.1
5	85	15	43.2	38.7	1.5	0.7	11.1	12.7	5.7	72.9	81.5
6	90	10	41.4	36.0	0.9	0.3	9.0	12.6	5.4	72.0	81.0

With data presented in Table 4, it was revealed that through all testing methods sphagnum shows its antiseptic activity. Furthermore, the streaking method and disk diffusion test were more effective against strains of *Staphylococcus aureus* and *Astinomyces sp.* Growth suppression of *Staphylococcus aureus* cultures was 45, 63.9, and 86.6 mm respectively. The assessment of sample antiseptic activity towards fungi of the *Candida*

spp. genus shows that the activity decreases by 2-3 times.

It was interesting to study the ability of antiseptic action of the made bandaging material in the process of long-term storage and under temperature. The test results are presented in Table 5. Antiseptic activity was tested towards *E.Coli* and *Bacillus cereus* microorganisms.

Table 5

The assessment of antiseptic activity of long-storage samples towards *E.Coli* and *Bacillus cereus*

Object under study	Microorganism growth suppression, mm	
	<i>E.Coli</i>	<i>Bacillus cereus</i>
Sphagnum (10-year-old)	13	10
Bandaging material	15	14
Fresh sphagnum	15	15
Dried sphagnum *	12	7

* drying was made at a temperature of 100-110°C for 3 hours.

According to the results of studies, it was revealed that the sensitivity of sphagnum over time (more than 10 years) towards the *E.Coli* strain does not weaken. Sphagnum is active towards this strain through the non-woven material as well as fresh moss. As for *Bacillus cereus* bacteria, samples after 10-year storage show slightly less

antiseptic activity towards this strain. Sphagnum drying is likely to lead to the removal of some low-boiling and more volatile compounds and, as a consequence, to the decrease in antiseptic activity of the bandaging material almost twice (Table 5).

The assessment of presence of trace elements in sphagnum is given in Table 6.

Table 6

Results of sphagnum X-ray spectral analysis method (according to Figure 1)

Metal samples	Relative metal content, %			
	upper part	middle part	lower part	whole stem
Mg	5.84	5.80	5.70	5.90
Al	3.50	3.45	3.6	3.60
Si	29.99	18.23	3.23	19.0
P	2.30	3.07	5.28	4.50
S	2.11	3.07	4.19	6.40
Cl	0.55	3.36	2.59	3.30
K	13.29	40.13	68.34	30.0
Ca	15.11	17.86	10.04	14.0
Ti	0.91	0.47	0.16	0.50
V	0.02	0.009	0.007	-
Cr	0.04	0.02	0.005	0.04
Mn	1.98	1.86	1.51	2.30
Fe	22.94	10.71	3.49	21.0
Ni	0.05	0.03	0.02	0.04
Cu	0.08	0.07	0.07	0.07
Zn	0.72	0.58	0.50	0.60
Ga	0.002	-	-	-
Se	-	-	0.001	-
Br	0.03	0.03	0.02	-
Rb	0.07	0.16	0.38	0.1
Sr	0.15	0.12	0.09	0.2
Ba	0.19	0.16	0.05	0.5
Pb	0.12	0.06	0.02	0.09

According to the data obtained, it has been found that sphagnum contains a large number of trace elements that contribute to wound healing. Such elements include phosphorus, silicium, aluminum, etc.

Conclusions:

1. Studies have shown that sphagnum contains a sufficient amount of substances potentially capable of antiseptic action on various conditionally pathogenic microorganisms.

2. The possibility of manufacturing new surgical bandaging materials with antiseptic properties and with high absorption capacity on domestic raw materials is shown.

3. It was found out that sphagnum contains a large number of trace elements, among which there are such elements as phosphorus, silicium, aluminum that contribute to the acceleration of regeneration of fresh tissues in wounds.

Conflict of interest. The authors declare that there is no conflict of interest.

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