02

Comparative assessment of the spectral characteristics and biological activity of acetone extracts of medical rubbers and their effect on the morphology of test culture colonies

© E.N. Riger,[1,2] I.M. Tsypkina,[1] A.Yu. Grozescu,[1] T.E. Sukhanova,[1] L.M. Popova[1,2]

[1] Federal State Budgetary Institution „S.V. Lebedev Institute of Synthetic Rubber"
198035 St. Petersburg, Russia
[2] Peter the Great Saint-Petersburg Polytechnic University,
194021 St. Petersburg, Russia
e-mail: catherine.riger@yandex.ru; tat_sukhanova@bk.ru

Received February 28, 2024
Revised June 26, 2024
Accepted June 26, 2024

Acetone extracts of various brands of natural and synthetic *cis*-1,4-isoprene rubbers were obtained by extraction using the Soxlet apparatus. Using a modified method for evaluating the biological activity of compounds, the effect of the prepared extracts of various concentrations in the range from 1 to 50 mg/ml on the size of the growth inhibition zone of test culture colonies was analyzed. Bacterial colonies were used to determine the antimicrobial properties of rubber extracts, and fungicidal activity was determined using mold fungi and yeast. It was shown by the method of IR- spectroscopy with DTIR that the extracts of the studied rubbers have different compositions and contain different amounts of stearic, oleic and linoleic acids, proteins and enzymes. The mathematical processing of the results, the calculation of the biological and fungicidal activity of the extracts were carried out using the PASS-online software package. A comparative assessment of the antimicrobial properties of synthetic and natural rubber extracts showed that extracts of synthetic isoprene rubber of the SKI-5 PM brand (PM — food medical) have the greatest inhibitory effect against yeast *Saccharomyces boulardii* CNCM I–745. It was also found that these extracts have a maximum growth suppression rate (1.2 ± 0.1 mm/day) of widespread *Aspergillus niger* mold fungi compared with extracts of natural rubbers. Thus, it is shown that for the production of medical and food industry products, imported natural rubbers can be replaced to a large extent by domestic synthetic analogues.

Keywords: natural and synthetic *cis*-1,4-isoprene rubbers, acetone extracts, IR-spectroscopy, biological activity, morphology of test cultures, antibacterial and fungicidal properties

DOI: 10.61011/TP.2024.09.59286.58-24

Introduction

The range of use of rubbers of various brands in the field of medicine is limited by the content of harmful impurities and/ or substances in them that have dangerous properties for the body, including carcinogenic ones. It was found, for example, that latex gloves made of natural rubber (NR) negatively affect human skin, a significant amount of proteins in latex provokes the development of allergies or eczema. In addition, NR may contain N-paranitrosoamines capable of causing oncological diseases [1]. The use of a catalytic system based on rare earth metals of the lanthanum group ensures the absence of oligomers in synthetic isoprene rubbers in their production, which makes synthetic rubbers (SR) safe for use in the medical and food industries.

New generation rubbers — „environmentally safe" rubbers — intended for use in the medical and food industries, are produced in Russia in accordance with specially developed specifications and are subject to mandatory certification [2]. At the same time, medical rubbers should have a wide range of biological activity [3]. Nevertheless, there is practically no literature data about the antimicrobial and fungicidal properties of rubbers of various origins.

It should be noted that the Russian SR production industry supplies 90% of domestic products for our country [4].

Currently, one of the main trends in the production of rubbers for medical and food purposes is the search for safe formulations that do not contain toxic oils and substances with carcinogenic properties. The issue of replacing NR by creating synthetic equivalents is extremely relevant for the domestic industry since it is not possible to grow rubber plants in sufficient quantities in our country [5]. Unlike SR, NR has a number of significant disadvantages, namely, NR from different manufacturers have variable composition and unstable properties due to climatic features, territory, method of extraction and some other factors [6].

The purpose of this paper was to obtain acetone extracts of medical rubbers, a comparative study and identification of the original rubbers, as well as extraction products extracted from them, using the IR spectroscopy method, and an assessment of their antimicrobial and fungicidal properties against bacterial cultures and micromycetes.

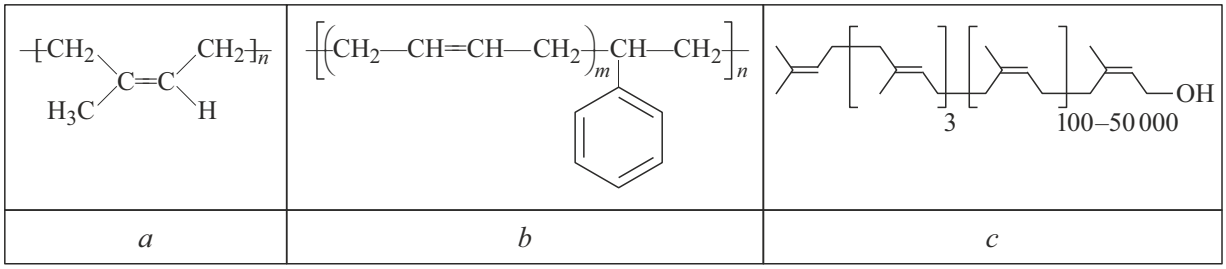

Figure 1. Structural formulas of the objects of research: *a* — structural formula of SKI-5 PM rubber (*cis*-1,4-isoprene); *b* — structural formula of SKMS-30 ARKM-27 rubber; *c* — the structural formula of NR.

The final stage of the study was to establish the possibility of replacing imported natural rubber in medical and food industry products with domestic synthetic equivalents.

It should be noted that the so-called acetone extract is a set of substances extracted by acetone from rubbers, it consists of oleic, stearic, linoleic acids, and their esters, phospholipids, carotene, etc. [5,6].

1. Experiment

1.1. Materials

Acetone extracts of the SR brands SKI-5 PM (*cis*-1,4-isoprene food medical rubber) and SKMS-30 ARKM-27 (butadiene-α-methyl styrene rubber, JSC „Sintez-Kauchuk“, Sterlitamak, Russia) were studied in the paper.

SKI-5 PM was obtained by polymerization of isoprene on a neodymium catalyst in accordance with TU 2294-051-16810126-2004 [7]. The content of *cis*-1,4-links is not less than 96%. The structural formula of *cis*-1,4-isoprene is shown in Fig. 1, *a*.

Styrene butadiene rubber SKMS-30 ARKM-27 is a product of low-temperature emulsion copolymerization of butadiene-1,3 and styrene being the most common type of general purpose rubbers [8]. The structural formula is shown in Fig. 1, *b*.

NR is *cis*-1.4 polyisoprene being an unique product of plant origin. The structure of NR molecules is shown in Fig. 1, *c*. NR is a biopolymer starting with a dimethylallyl group, followed first by three monomeric residues with a trans-C=C bond, followed by 100 to 50,000 monomeric residues with a it cis-C=C bond, and the terminating hydroxy group is located at the end of the molecules [5]. 98−100% of the isoprene links are attached in the position *cis*-1.4 in the NR macromolecule.

They were the main raw material for the production of rubber products with a high degree of elasticity until the early 30s of the last century. And currently, NR from hevea [5] is used in the production of high-quality and special tires and rubber products, including for military equipment. Moreover, NR belongs to low-toxic materials, which causes its widespread use in food and medical products [3,4].

NR is formed in the course of the vital activity of rubber-bearing plant cells, and latex is the cytoplasm for the biosynthesis of rubber [5]. The uniqueness of the properties of NR is attributable to the presence of non-rubber components in its composition in addition to *cis*-1,4-isoprene (up to 96 weight%), namely natural protein fragments chemically bound to macromolecules of biopolymer, acetone extract, and some other components [5]. It is known that latex contains a protein (up to 5 weight%) [5], which has a significant effect on the properties of NR. Part of the protein is deposited together with rubber in the process of separation of NR from latex, together with the denaturation process. It was found in Ref. [9] using the method of electrophoretic separation of rubber proteins from a number of plants (SDS-gel electrophoresis), that NR from hevea contains more than 15 major and minor proteins. Calculations of the ratio of proteins to rubber showed that hevea contains more than 9 mg of proteins per 1 g of dry weight of rubber.

Two natural rubbers of the brands CVL-20 and SVR-10 (Viet Phu Thinh Rubber Joint Stock Company, Vietnam) with different impurity contents (10 and 20 weight%, respectively) were selected as comparison objects for estimation of the biological activity of acetone extracts of rubbers of various origins. Groups of rubbers of grades SV and CV — standard grades with constant viscosity, L — grades with reduced coloration.

1.2. Preparation of rubber extracts

The extraction was performed using device Soxlet ASV−6M (LLC „Vilitek“, Russia) according to GOST ISO 1407-2013 [10]. This device allows extraction with periodic washing of the sample with a certain solvent. Acetone was used as a solvent in accordance with GOST 24919-91 [11]. The degree of purity of the acetone used (99.9%) was checked by gas chromatography on using Clarus 500 system (PerkinElmer, USA). Each sample of rubber weighing $m_n = 3.00 \pm 0.01$ g was dissolved in 50 m*l* of acetone. The extraction was carried out for 16 h.

Acetone removal from the obtained extract was performed using rotary evaporator model EV311VAC (Prime-Lab, Russia) at $60 \pm 5^\circ$C.

Table 1. Names and characteristics of test cultures

Name of microorganisms	Type of microorganisms	Pathogenicity
Escherichia coli	Gram-negative rod-shaped bacteria	Non-pathogens, present in the lower intestine of warm-blooded animals and humans, can cause severe food poisoning
Bacillus subtilis	Gram-positive spore-forming aerobic soil bacteria	Non-pathogens, used in a number of medicines and products for veterinary and agriculture
Alternaria alternata	A type of spore-forming (ascomycete, marsupial) mold fungi	Pathogens of plants, animals and humans (cause allergies), spores are present in soil, water and on the surface of technical facilities and premises
Aspergillus niger	A kind of higher mold fungi, belongs to the class of marsupial fungi (*Ascomycetes*)	Strong pathogens (penetrate into the body and affect the respiratory and central nervous system, digestive tract, skin, sensory organs and reproductive system)
Penicillium spp	A kind of fungi from the class of deuteromycetes or imperfect fungi (*Deuteromycota*)	Weak pathogens that live in soil and on the surface of substrates of plant origin
Rhodotorula rubra	Asporogenic yeast fungi belonging to the family *Cryptococcaceae*	Pathogens, fungi can be present on the skin and nails as a saprophyte, can colonize the respiratory and urinary tracts
Candida tropicalis	Yeast-like fungi that cause urogenital candidiasis	Pathogens are part of the normal microflora of the mouth, vagina and colon of most healthy people
Saccharomyces boulardii CNCM I-745	Type of yeast	Non-pathogens, produce proteins that inhibit pathogenic bacteria and their toxins

1.3. Test microorganisms

Microorganisms used to determine the antibacterial properties of these reagents are listed in Table 1.

Cultures of fungi and bacteria were provided by the Museum of the Department of Microbiological Synthesis Technology of SPbGTI (TU).

1.4. Research methods

1.4.1. Biological testing of rubber extracts

The qualitative disco-diffusion test method (DDT) for determining antibacterial activity is based on methodological guidelines describing standard methods for determining the sensitivity of microorganisms to antibacterial drugs — methods of serial dilution and DDT (MUK 4.2.1890−04 „Determination of the sensitivity of microorganisms to antibacterial drugs“) [12].

A sterile paper disk with a diameter of 1.5 cm, made of filter paper, was used in DDT as a carrier of the studied reagent — acetone extract of rubber. One paper disk preimpregnated with the test extract solution in the amount of 0.1 μm was inserted into the center of the Petri dish. Disks moistened with a solvent (distilled water) were used as a control. Then the Petri dishes were placed in a thermostat for the cultivation of test microorganisms, the cultivation conditions are given in Table 2. The diffusion of the studied extract from the disk into the culture medium resulted in the formation of a zone of suppression (inhibition) of the growth of test microorganisms. Recording of the diameter of the growth inhibition zone around the disk made it possible to determine the biological activity of the drug.

The zone of suppression of the growth of microorganisms was determined using a measuring ruler. All experiments were carried out in three repetitions.

Dry culture media (Table 3) were used in this study: culture medium No 2 GRM (Saburo) and hexametaphosphate agar (GMP-agar), the advantages of which are high sensitivity, long shelf life and ease of preparation.

The culture medium was prepared in a non-standard way: after preparation, the culture medium was poured in 10 m *l* test tubes, an extract of a certain concentration was introduced, the tubes were capped and boiled in a water bath 2−3 times for 40 min. The contents of the test tubes were then poured onto the surface of the Petri dish. Mold fungi spores were inoculated by injection with a needle after solidification of the culture medium. The degree of impact of the studied extracts on mold fungi was

Table 2. Types of microorganisms used and conditions of their cultivation

Names of Used microorganisms	Method of study	Culture medium	Temperature of Incubation, °C	Duration of incubation, h
Yeast				
Candida tropicalis	DDT	Saburo	33	48
Rhodotorula rubra				
Saccharomyces boulardii CNCM I-745				
Bacteria				
Escherichia coli	DDT	GMF	37	24
Bacillus subtilis				
Fungus				
Alternaria alternate	Fungus colony size measurements in case of growing on a culture medium, in which the extract of a certain concentration was added	Saburo	26–28	192
Aspergillus niger				72
Penicillum spp.				120

Table 3. Characteristics of the culture media used

Name	„Culture medium №2 GRM (SABURO)" according to TU 9398-002-78095326-2006	GMF-agar
Description	Fine Hygroscopic Powder	Nutrient agar for the cultivation of microorganisms, dry on the basis of beef enzymatic hydrolysate, is a finely dispersed, hygroscopic yellow powder
Composition, g/*l*	Pancreatic hydrolysate of fish meal — 10.0; pancreatic hydrolysate of casein — 10.0; yeast extract — 2.0; monosubstituted sodium phosphate — 2.0, glucose — 40.0, microbiological agar — 7.0	GMF-base — 15.0; sodium chloride — 9.0; microbiological agar — from 12.0 to 15.0
Purpose	For the cultivation and counting of the total number of yeast and mold fungi in the control of microbial contamination of non-sterile medicines	For the isolation, cultivation and identification of various microorganisms, including: Enterobacteria, Pseudomonas aeruginosa, staphylococci

evaluated in this paper using the method of determining the size of the fungus colony on a culture medium into which the extract was previously introduced.

1.4.2. IR spectroscopic studies of rubber extracts

IR spectra were recorded using Spectrum 100 infrared Fourier spectrometer (PerkinElmer, USA) with an attenuated total reflection (ATR) accessory with a diamond crystal. Spectral recording range $4000-600\,cm^{-1}$, resolution — $0.5\,cm^{-1}$, number of scans — 4. The samples were held under an IR lamp for a day before recording the IR spectra to remove the solvent, then the spectra of dried rubber extracts and starting rubbers were acquired.

1.4.3. Method of calculation of biological activity in the PASS-online program

PASS-online program was used to predict the spectrum of probable biological activity of the components that make up natural and synthetic rubbers. This program has been functioning since 2000 as a freely available web resource [13], in which the description of the structure of organic compound molecules is implemented by MNA (Multilevel Neighborhoods of Atoms) descriptors and an algorithm based on the „naive Bayesian approach". This program allows identifying structure–activity relationships between classes of various active and inactive compounds. The developers of the program note that the PASS-online prediction algorithm is characterized by statistical stability, which ensures the most likely estimates of the biological activity spectra for new compounds. The modern version of

Table 4. Zone of inhibition of growth of *Candida tropicalis yeast* with rubber extracts (DDT)

Samples	Extract concentration, mg/m*l*					
	0.1	1	5	10	30	50
	Size of the inhibition zone, mm					
Extract of NR SVR-10	–	–	–	1.5 ± 0.2	3.0 ± 0.4	5.0 ± 0.3
Extract of SR SKI-5 PM	–	2.0 ± 0.5	5.0 ± 0.4	6.0 ± 0.4	7.5 ± 0.4	8.5 ± 0.5
Extract of NR CVL-20	4.0 ± 0.2	6.0 ± 0.3	8.0 ± 0.3	8.5 ± 0.4	10.0 ± 0.7	11.0 ± 0.3
Extract of rubber SKMS-30 ARKM-27	10.0 ± 0.5	11.0 ± 0.3	11.0 ± 0.5	10.0 ± 0.5	10.0 ± 0.6	10.5 ± 0.3

the PASS-online computer program allows predicting more than five thousand types of biological activity.

2. Results and discussion

Medical and food-grade rubbers based on natural and synthetic rubbers approved by health authorities for the manufacture of medical devices or in contact with food should have a set of specific properties due to their purpose, including a wide range of biological activity [3].

2.1. Study of fungicidal properties of acetone rubber extracts

2.1.1. Evaluation of fungicidal properties of rubber extracts in relation to yeast test cultures

The SR extracts with concentrations ranging from 0.1 to 50 mg/m*l* demonstrated the best results in inhibiting the growth of yeast *Candida tropicalis,* with specific characteristics (the cell wall includes chitin and glucan, which affect the structure of the yeast cell [14]). The dose-dependent activity of the obtained extracts against the test cultures used is observed almost in all cases (Table 4). The largest zone of inhibition of *Candida tropicalis* yeast for extract SKMS-30 ARKM-27 corresponded to a concentration equal to 1 mg/m*l*. Further, no growth of the inhibition zone was observed with an increase of the concentration of the extract.

The images in Fig. 2, *a, b* confirm that the concentration values of the rubber extract have a significant effect on the growth of the test strains. Thus,*Candida tropicalis* yeast culture forms a more pronounced growth inhibition zone with an increase of the concentration of SKI-5, PM extract — the size of the zone increases by about 4 times when the concentration of the extract changes from 1 to 50 mg/m*l* (Table. 4), which is attributable to the presence of impurities in the extract with anti-candida properties [15]. The maximum size of the inhibition zone of the order 11 mm was found in the case of usage of the extract of rubber SKMS-30 ARKM-27, and the size of the zone does not depend on the concentration of the extract. This may be caused by the high content of TDAE plasticizer oil in this rubber. The control sample contains no rubber extract (Fig. 2, *c*).

The images (Fig. 2, *d, e*) show isolated budding fungi *Saccharomyces boulardii.* An increase of the culture growth inhibition zone indicates that the rubber extract SKI-5 PM has fungicidal properties (Tables 5, 6). The control solution contains no extract (Fig. 2, *f*).

Rhodotorula rubra yeast is usually found on the surface of the skin and mucous membranes of the human body, they are easily identified visually by pronounced orange colonies (Fig. 2, *h*, control). As our microbiological and morphological studies have shown, extracts of both NR and SR exhibit fungicidal properties with respect to this strain, since the culture growth is completely inhibited when extracts of various rubbers are added (Fig. 2, *g*).

A comparison of the results obtained by different methods shows that DDT is more explicit for determining the microbiological activity of compounds and the same dependence is observed based on the data presented in Table. 5 and 6: the inhibition zone of *Saccharomyces boulardii CNCM I-745* yeast decreases by 2 times with an increase of the concentration of NR extract from 1 to 50 mg/m*l*.

On the contrary, the inhibition zone in the same concentration range also increases by 2 times with an increase of the concentration of the SR extract, i.e. it was shown based on *in vitro* results that acetone extracts of various brands of rubbers exhibit antimicrobial and fungicidal activity against *Saccharomyces boulardii CNCM I-745* yeast.

2.1.2. Evaluation of fungicidal properties of rubber extracts in relation to mushroom test cultures

The images (Fig. 3, *a, b*) show „overgrown" Petri dishes with *Aspergillus niger* — the most studied and widespread culture in everyday life, belonging to the class of cup fungi (*Ascomycetes*). It is known [16,17] that the body of the fungus consists of colorless, highly branched and intertwined thin filaments−hyphae forming mycelium (mycelium). The hyphae are separated by transverse partitions (septa) into cells. The diameter of the hyphae usually ranges from 3 to 6 μm. This culture is very resistant to environmental impacts. Colonies of „black mold" on walls of damp rooms — these are mainly *Aspergillus niger* in the

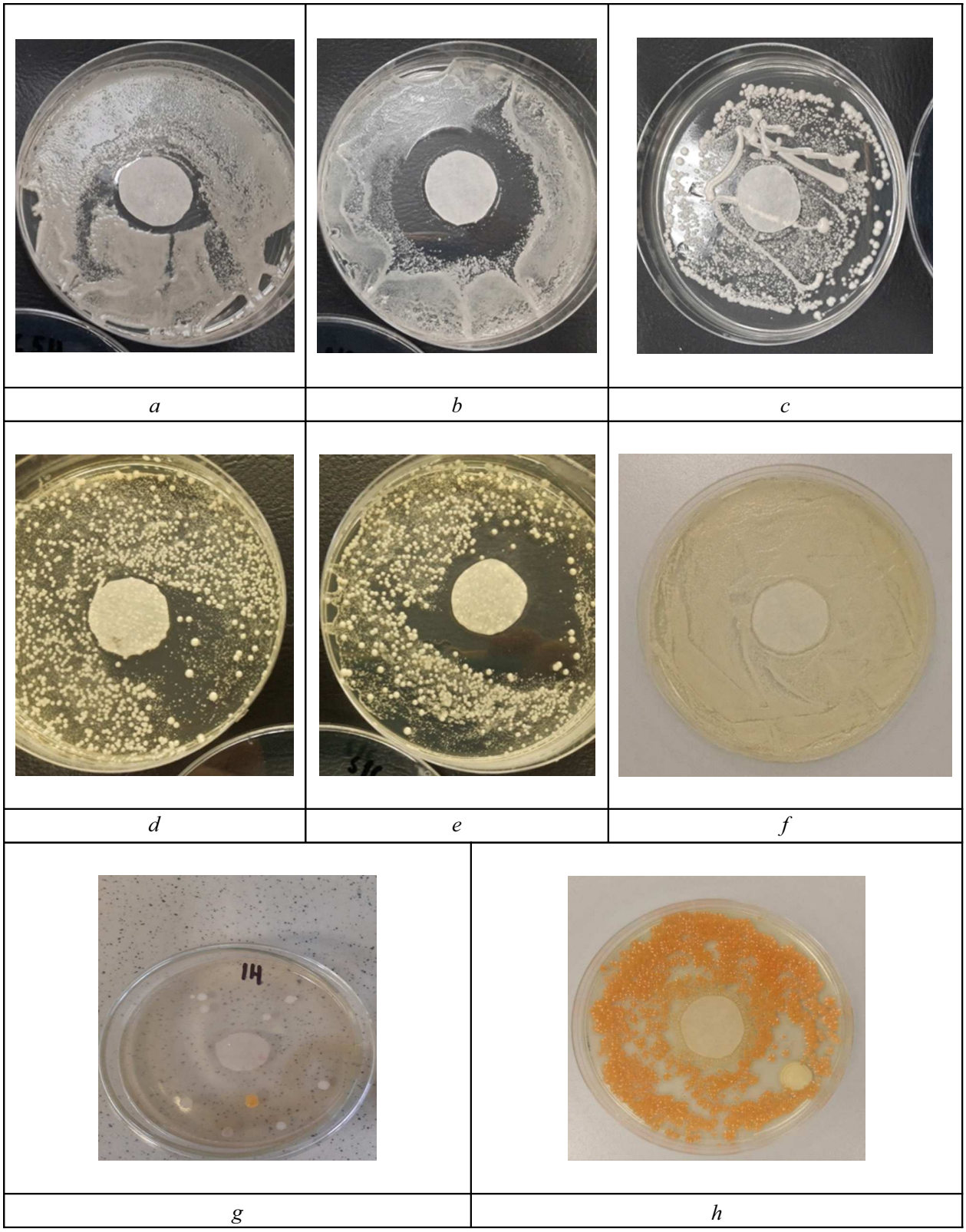

Figure 2. The effect of the obtained extracts of rubber SKI-5 PM on *Candida tropicalis* yeast culture: *a* — solution with an extract concentration of 5 mg/ml; *b* — solution with an extract concentration of 50 mg/ml; *c* — control. The effect of the obtained extracts of rubber SKI-5 PM on *Saccharomyces boulardii CNCM I-745 culture: d* — solution with an extract concentration of 5 mg/ml; *e* — solution with an extract concentration of 50 mg/ml; *f* — control. The effect of extract of rubber SKI-5 PM on *Rhodotorula rubra culture: g* — solution with an extract concentration of 5 mg/ml; *h* — control. All measurements were performed after 48 h.

Table 5. Zone of inhibition of growth of *Saccharomyces boulardii CNCM I-745* yeast colonies by rubber extracts („injection“ method)

Samples	Extract concentration, mg/m*l*		
	5	20	30
	Size of the inhibition zone, mm		
Extract of NR SVR-10	2.0 ± 0.5	1.0 ± 0.5	–
Extract of SR SKI-5 PM	–	2.0 ± 0.3	3.0 ± 0.5

Table 6. Zone of inhibition of growth of *Saccharomyces boulardii CNCM I-745* yeast colonies by rubber extracts (DDT)

Samples	Extract concentration, mg/m*l*						
	0.1	1	5	10	20	30	50
	Size of the inhibition zone, mm						
Extract of NR SVR-10	9.5 ± 0.5	9.0 ± 0.2	8.5 ± 0.5	8.0 ± 0.6	7.0 ± 0.8	5.0 ± 0.2	4.0 ± 0.5
Extract of SR SKI-5 PM	3.0 ± 0.3	4.5 ± 0.6	5.0 ± 0.4	5.0 ± 0.5	6.0 ± 0.6	7.0 ± 0.4	8.5 ± 0.6
Extract of NR CVL-20	7.0 ± 0.5	9.0 ± 0.6	8.0 ± 0.3	10.0 ± 0.5	11.0 ± 0.4	11.5 ± 0.4	10.0 ± 0.5
Extract of SR SKMS-30 ARKM-27	7.0 ± 0.4	7.5 ± 0.1	8.0 ± 0.4	11.0 ± 0.1	12.0 ± 0.3	12.5 ± 0.4	13.0 ± 0.7

Table 7. The size of the colony of *Aspergillus niger* fungi depending on the concentration of rubber extracts („injection“ method)

Samples	Extract concentration, mg/m*l*				
	0.1	1	5	20	30
	Colony size, mm				
Extract of NR SVR-10	3.0 ± 0.5	5.0 ± 0.4	6.0 ± 0.4	14.0 ± 0.4	15.0 ± 0.6
Extract of SR SKI-5 PM	9.0 ± 0.3	11.0 ± 0.3	14.0 ± 0.6	16.0 ± 0.5	19.0 ± 0.6
Extract of NR CVL-20	8.0 ± 0.5	10.0 ± 0.5	13.0 ± 0.6	17.0 ± 0.1	20.0 ± 0.5
Extract of SR SKMS-30 ARKM-27	4.0 ± 0.4	7.0 ± 0.5	10.0 ± 0.3	14.0 ± 0.4	18.0 ± 0.8

Table 8. Dynamics of growth of *Aspergillus niger* fungal colony („injection“ method)

Samples	Colony diameter, mm		
	3 days	7 days	10 days
SKI-5 PM ($C = 0.5$ mg/m*l*)	20	30	52
SKI-5 PM ($C = 3$ mg/m*l*)	18	30	50
NR SVR-10 ($C = 2$ mg/m*l*)	27	48	52
NR SVR-10 ($C = 3$ mg/m*l*)	29	45	56

fruitage phase, their damaging effect is associated with the production of large amounts of organic acids. These colonies are extremely dangerous because the fungus can penetrate and settle in the human lung cavity and cause a fungal disease called „pulmonary aspergillosis“ [16,17].

Observations of the growth dynamics of mold fungi were carried out for 10 days in order to determine the growth rate on the Saburo culture medium and assess the effect of NR and SR extracts on the growth of test strains (Tables 7, 8). The composition of the Saburo medium ensures clear morphological features. Spore-forming fluffy colonies have a „compact“ localization and have different color shades — from white to dark brown. Mycelium forms partitions, forming numerous „sites“ with a high density of „colonization“ with a diameter from 3 to 11 mm (Fig. 3, *a, b*). In turn, the control sample is characterized by the radial character of the growth of spore-forming units (Fig. 3, *c*).

An interesting dependence is observed in Fig. 3, *d, e*, namely, the color of folded convex colonies of *Penicillium spp.* changes with an increase of the concentration of the extract of SKI-5 PM. Colonies on the control sample without the addition of extract (Fig. 3, *f*) have a dark green color, a solution with the addition of a minimum

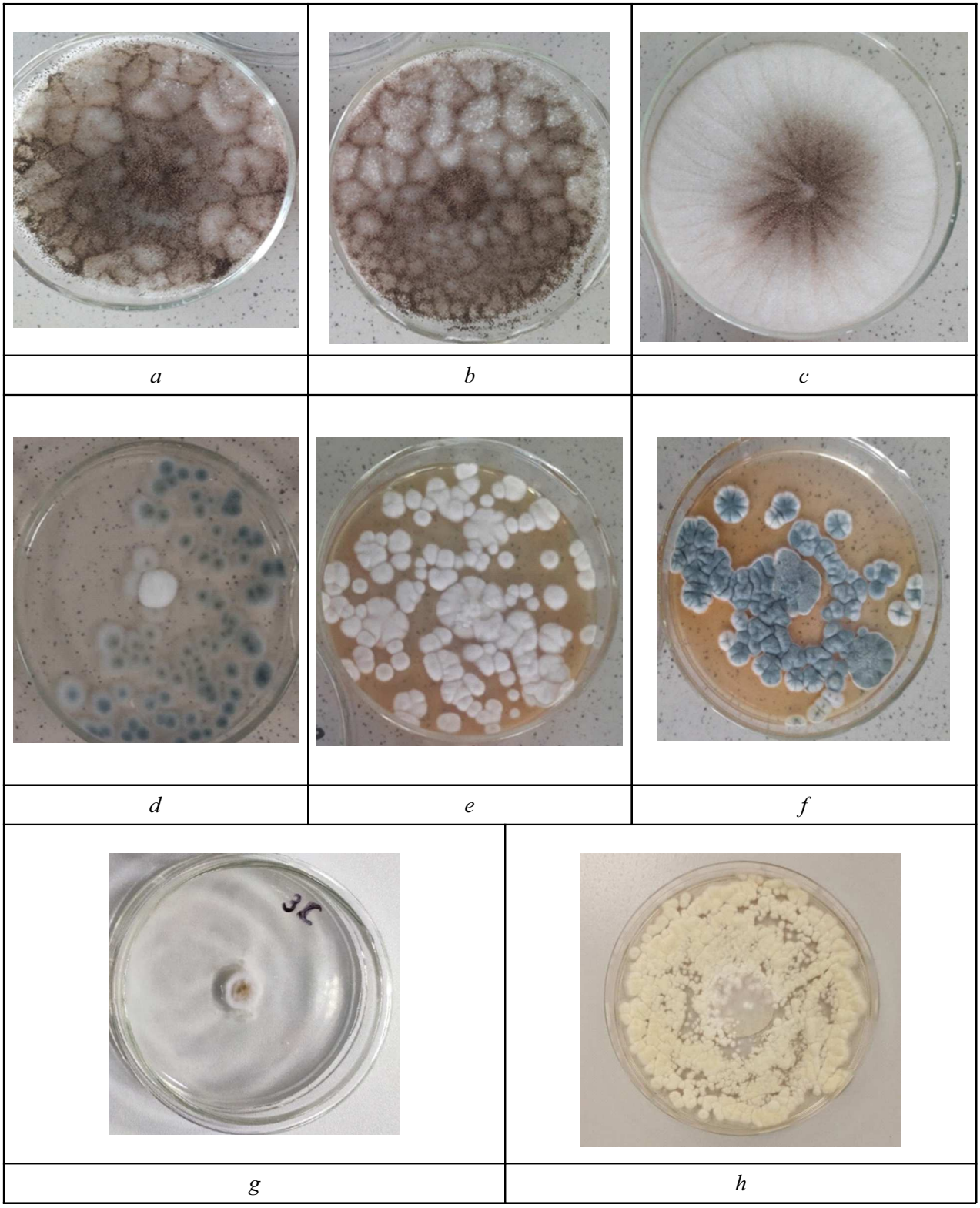

Figure 3. The effect of the obtained extracts of rubber SKI-5 PM on *Aspergillus niger* culture (measurement was performed after 72 h): *a, b* — solutions with an extract concentration of 30 mg/ml; *c* — control. The effect of the obtained extracts of rubber SKI-5 PM on *Penicillium spp.* culture (measurement was performed on 5th day): *d* — solution with an extract concentration of 1 mg/ml; *e* — solution with an extract concentration of 5 mg/ml; *f* — control. Effect of the extract of rubber SKI-5 PM on *Alternaria alternata* culture (measurement was performed on 8th day): *g* — solution with an extract concentration of 0.1 mg/ml; *h* — control.

concentration (1,mg/ml) of extract of SKI-5 PM (Fig. 3, *d*) has a green color, and the color changes to white with the increase of the concentration (starting from 5 mg/ml) (Fig. 3, *e*). Morphological changes are also observed at the same time: fungal colonies become more isolated.

Fig. 3, *g* shows an optical photograph of a Petri dish with the addition of a minimum concentration (0.1 mg/ml) of the extract of SKI-5 PM. The „injection" method allowed determining that the growth rate of velvet colonies of *Alternaria alternata* is low.

The results provided in Table. 7 and 8 show that dose-dependent activity is observed for all NR and SR extracts. The extract of synthetic rubber SKI-5 PM has a maximum rate of inhibition pf growth (1.2 ± 0.1 mm/day) of widespread mold fungi *Aspergillus niger* compared with extracts of NR (0.8 ± 0.2 mm/day). This pattern is probably related to the presence of sugars, proteins and enzymes in the NR composition, which create good conditions for the development of various types of mold.

A comparison of photos of cultures in Fig. 3, *d–f* indicates that the introduction of the obtained rubber extracts suppresses the growth of the test culture of *Penicillium spp.,* belonging to the class of imperfect fungi of (*Deuteromycota*). The habitat of this crop is soil and substrates of plant origin. The morphological feature of *Penicillium spp.* is that they form a mycelium of separated branching hyphae, at the ends of which primary and secondary branching — metules I and II-th order (multi-lobed tassels) are formed. Bundles of bottle-shaped phialides radiate from the tops of the metules, bearing chains of rounded conidia of green, yellow-brown, pink or purple [17].

Convex colonies form a branched mycelium on the dense culture medium Saburo, on which special hyphae bearing spores are developed. An increase of the concentration of NR and SR extracts actively inhibit the spread of colonies, as evidenced by the size of the inhibition zone (Table 9).

Extracts of natural and synthetic rubbers equally exhibit antimicrobial activity against *Alternaria alternata fungi:* an increase of the size of the colony of microorganisms is observed with an increase of the concentration of extracts (Tables 10, 11).

Morphology, dimensional characteristics and fine structure of colonies of *Aspergillus niger* and *Penicillium spp.* were studied in sufficient detail in Ref. [17–20]. The scheme of the structure of *Aspergillus niger* and *Penicillium spp.* fungi is shown in Fig. 4 [20].

It was found that the vegetative body of *Aspergillus niger* is a very branched mycelium penetrating the substrate. Conidiophores which are hyphae of mycelium of fungi on which spores develop — move upwards from special mycelial cells which are supporting cells. The upper part of the conidiophore swells, forming a bubble. There are flag-shaped cells on the bubble or only in its upper part — phialids, from the narrow neck of which single-celled conidia radially emerge one after another, arranged in a chain. Mature conidia have a spherical shape and become spiny or tuberculate in *Aspergillus niger* upon maturation

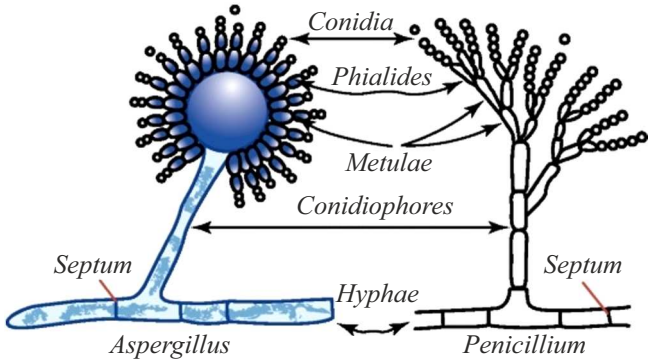

Figure 4. Schematic representation of the structure of *Aspergillus niger* and *Penicillium spp.* fungi [20].

(Fig. 4) [20]. The coloration of the conidia mass gives a different color to the mold plaque (colonies) [18]. In our case, the observed conidia have sizes from 4 to 6 μm in diameter (Fig. 3, *a–c*) and a dark brown color.

In turn, *Penicillium spp.* have a well-developed branched multicellular mycelium, they are reproduced mainly by conidial sporulation. Filamentous hyphae (diameter of hyphae — 2−3 μm) form the main structure of septic, i.e. separated by partitions, conidiophores *Penicillium spp.* Branched sections of conidiophores have one whorl of phialides at the top, they can also have a two-tiered structure and consist of metules and phialides located on them (Fig. 4) [20]. It should be noted that, according to the literature data [19], they may have different colors — white, green, etc. depending on the type of conidia.

It was shown in our experiments that the conidia of *Penicillium spp.* colonies have different colors depending on the concentration of the extract of SKI-5 PM (Fig. 2, *d–f*), colonies reach 3−4.5 μm in diameter In addition, the distance between colonies changes depending on the concentration.

The results of the experiments show that it is safer to use synthetic equivalents of NR as materials for the manufacture of rubber products used in everyday human life. The reason is the active growth of mold fungi on the surface of rubber products based on NR.

2.2. Study of antibacterial properties of acetone rubber extracts

Bacteria *Bacillus Subtilis* — gram-positive spore-forming aerobic bacteria, widespread in soil, having a damaging effect on the materials they come into contact with.

The culture of bacteria *Bacillus Subtilis* was seeded into Petri dishes under sterile conditions using a bacterial loop, a Drygalsky spatula was used to evenly distribute the culture. A sterile paper disk ($d = 1.5$ cm) made of filter paper impregnated with the extract of SKI-5 PM was placed in the center of the Petri dishes.

The formation of a pronounced zone of inhibition of the growth of bacterial culture was observed under the

Table 9. Zone of inhibition of growth of fungus *Penicillium spp.* by rubber extracts („injection"method)

Samples	Extract concentration, mg/m*l*					
	0.1	1	5	10	20	30
	Colony size, mm					
Extract of NR SVR-10	25.0 ± 0.5	25.0 ± 0.3	24.0 ± 0.3	24.0 ± 0.4	22.0 ± 0.3	16.0 ± 0.5
Extract of rubber SKMS-30 ARKM-27	19.5 ± 0.6	19.0 ± 0.5	18.0 ± 0.7	16.0 ± 0.5	15.0 ± 0.6	13.0 ± 0.7
Extract of NR CVL-20	7.0 ± 0.8	13.0 ± 0.3	17.0 ± 0.3	18.0 ± 0.5	19.0 ± 0.7	19.5 ± 0.6
Extract of rubber SKMS-30 ARKM-27	6.0 ± 0.4	17.0 ± 0.2	18.0 ± 0.4	21.0 ± 0.2	23.0 ± 0.2	24.0 ± 0.5

Table 10. Zone of inhibition of growth of *Alternaria alternata* fungus by rubber extracts („injection" method)

Samples	Extract concentrations, mg/m*l*				
	0.1	1	5	10	50
	Colony size, mm				
Extract of NR SVR-10	2.0 ± 0.2	6.0 ± 0.5	8.0 ± 0.2	$C12.0 \pm 0.3$	15.0 ± 0.5
Extract of rubber SKI-5 PM	7.0 ± 0.8	9.0 ± 0.7	10.0 ± 0.1	11.0 ± 0.6	13.0 ± 0.7
Extract of NR CVL-20	–	10.5 ± 0.4	11.0 ± 0.6	12.0 ± 0.2	14.0 ± 0.7
Extract of rubber SKMS-30 ARKM-27	9.0 ± 0.6	10.5 ± 0.3	11.0 ± 0.4	11.5 ± 0.1	12.0 ± 0.4

Table 11. Dynamics of growth of colonies of *Alternaria alternata* fungi („injection" method)

Samples	Colony diameter, mm		
	3 days	5 days	7 days
SKI-5 PM (C = 0.5 mg/m*l*)	3	10	15
SKI-5 PM (C = 3 mg/m*l*)	5	8	16
NR SVR-10 (C = 2 mg/m*l*)	7	14	> 20
NR SVR-10 (C = 3 mg/m*l*)	6	12	19

impact of the extract, the size of the zone ranged from 3 to 5.5mm, which allowed evaluating the biological activity of the studied compound — acetone extract of SKI-5 PM (Table 12).

The morphology of the bacterial film were observed with an increase of the concentration of the extract, while the distribution of the culture over the area of the Petri dish acquired a looser character — almost half of the sown area was occupied by islet formations. A control solution containing no rubber extract was used for a comparative assessment of biological activity. At the same time, a uniform growth of colonies of microorganisms was observed with the formation of a homogeneous biofilm.

The sizes of the zone of inhibition of the growth of gram-positive bacteria *Bacillus subtilis* by rubber extracts determined by DDT are listed in Table 12.

The highest activity of NR and SR extracts against the *Bacillus Subtilis* strain was observed in case of usage of the minimum concentration of 5 mg/m*l*. The size of the inhibition zone decreases with an increase in the concentration of NR and SR extracts, and the antibacterial properties become less pronounced.

Various test cultures were selected, including both gram-positive bacteria *Bacillus Subtilis* and gram-negative bacteria *Escherichia coli* for comparing the spectrum of action of rubber extracts. The antibacterial properties of the extract of NR SVR-10 were studied with a variation of the concentration of the extract from 5 to 50 mg/m*l*. It was shown that at the same time the zone of inhibition of the growth of bacteria *Escherichia coli* increases, i.e. dose-dependent activity is observed.

The growth of the zone of inhibition of it Escherichia coli was observed only at concentrations above 30 mg/m*l* in case of extract of NR CVL-20.

In turn, SR extracts at minimal concentrations (1–10 mg/m*l*) showed no activity against the studied strain, and the growth inhibition zone was insignificant at high concentrations (30–50 mg/m*l*) — 3–5 mm.

2.3. Study of the spectral characteristics of rubbers and their acetone extracts by IR spectroscopy

Samples of original rubbers SKI-5 PM and SVR-10 as well as their acetone extracts were studied by IR spectroscopy using the ATR accessory.

Table 12. Zone of inhibition of gram-positive bacteria *Bacillus subtilis* by rubber extracts (DDT)

Samples	Extract concentration, mg/ml		
	5	20	30
	Size of the inhibition zone, mm		
Extract of NR SVR-10	6.5 ± 0.5	5.5 ± 0.4	2.0 ± 0.8
Extract of SR SKI-5 PM	5.5 ± 0.4	4.0 ± 0.3	3.0 ± 0.5

The spectra of *cis*-1,4-isoprene rubbers should have 5 characteristic absorption bands according to GOST 28665-90 (ISO 4650-84) [21]: 885 (very strong), 1370 (strong), 800 (medium), 1640 (medium) and 909 cm^{-1} (shoulder). The spectrum of the original rubber SKI-5 PM in Fig 5 (curve *1*, red) has the following absorption bands: 834 (deformation vibrations of CH groups); 1376 (deformation vibrations of CH_3 groups); 1645 cm^{-1} (valence vibrations C=C). In addition, three fairly intense bands are observed in the 3200–2500 cm^{-1} region — 2850, 2920 and 2960 cm^{-1}, which are related to valence vibrations of C–H bonds in CH_2 groups. These bands can be detected in the spectra of all compounds containing the hydrocarbon chain [22].

Figure 5 also shows the IR spectrum of the dried extract of rubber SKI-5PM (curve *2*, blue). It can be seen that absorption bands are present in this spectrum: 963 (deformation vibrations C–H (1,4-trans)); 1080 (deformation vibrations C–H); 1187, 1461 and 1495 (deformation vibrations CH_2); 2849 and 2921 cm^{-1} (valence asymmetric vibrations CH_2). It should be noted that the following changes occur in the extract spectrum: a doublet appears at 1740 and 1712 cm^{-1} and a number of narrow absorption bands of medium intensity in the region of 1500–850 cm^{-1}, and splitting into bands of 720 and 732 cm^{-1} also takes place. This is typical for substances having more than four CH_2 groups in the hydrocarbon chain and that have a solid state [22].

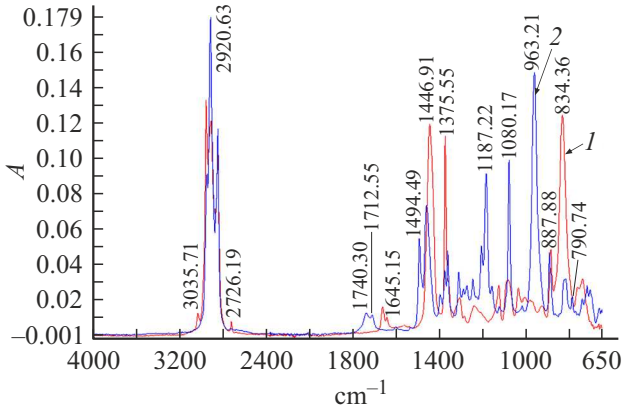

Figure 5. IR spectra of samples of rubber SKI-5 PM (*1* (red curve) — original rubber, *2* (blue curve) — dried rubber extract).

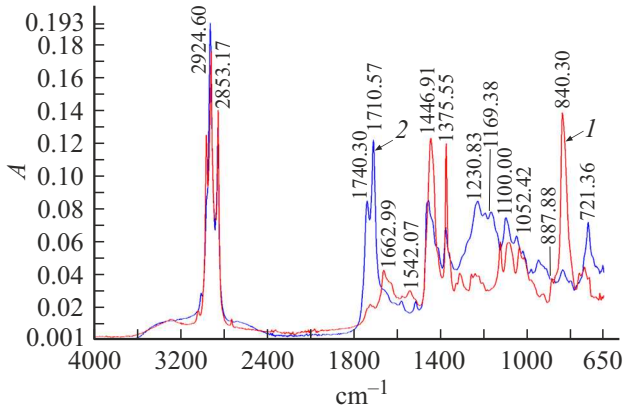

Figure 6. IR spectra of samples of natural rubber SVR-10 (*1* (red curve) — original rubber, *2* (blue curve) — dried rubber extract).

A comparison of the obtained spectra of synthetic rubber SKI-5 PM with the IR spectra of NR SVR-10 shows a significant difference both in the case of the original rubber and in the case of the extract.

The following intense absorption bands are present in the spectrum of original NR SVR-10 (Fig. 6, curve *1*, red): 840 — deformation vibrations of CH-groups; 1376 (deformation vibrations of CH_3-groups); 1447 cm^{-1} (deformation vibrations of CH_2-groups) and three closely spaced bands 2853, 2925 and 2960 cm^{-1} (valence vibrations of CH_3 methylene groups). In addition, bands of medium intensity are observed: 1663 (valence vibrations C=C), 1128–1017 cm^{-1} (deformation vibrations of CH-groups) and weak bands: 1542 (deformation vibrations of N–H-groups, proteins), 1312–1205 cm^{-1} (deformation vibrations of CH_2-groups).

Figure 6 shows the IR spectrum of the dried extract of NR SVR-10 (curve *2*, blue). It can be seen that absorption bands 721 — deformation vibrations CH_2-groups, 1052 — deformation vibrations of CH-groups (1,2); 1100, 1169 cm^{-1} — deformation vibrations of CH-groups, 1376 cm^{-1} (deformation vibrations of CH_3-groups), 1459 cm^{-1} with two arms (1443 and 1413 cm^{-1}) — deformation vibrations CH_2-groups; 2925 cm^{-1} (valence asymmetric vibrations CH_2). Moreover, a sharp increase of the intensity of the band was detected in this spectrum at 1740 cm^{-1} as well as the appearance of an intense band of

1711 cm^{-1}. This is attributable to the valence fluctuations of the carbonyl group (C=O) of stearic and oleic acids [22].

The spectrum of the original rubber SVR-10 in Fig. 6 also shows a broad „bell-shaped" peak in the range from 3600 to 3000 cm^{-1} with a peak at 3350 cm^{-1}, corresponding to the valence vibrations of N−H-groups (proteins) [23]. The extract spectrum has a broader band in the range from 3600 to 2400 cm^{-1}, which indicates that the extract contains other components of natural rubber (fatty acids, phospholipids, sugars, etc.) in addition to proteins [23].

2.4. Prediction of the spectrum of biological activity in the PASS-online program

The calculation was performed using the PASS-online software package for analyzing the results obtained and predict the biological activity of various components of SR and NR in this paper. The calculation results are listed in Tables 13, 14.

It turned out that the components of the SR are highly likely to have a carminative effect (for limonene — 96.1%) and exhibit the activity of an aspulvinondimethylallyltransferase inhibitor (for isoprene — 95.0%).

Table 13. Prediction of the biological activity of extracts of *cis*-isoprene SR using the PASS-online software package

Activity	P_a, %	P_i, %
Limonene		
Carminative	96.1	0.1
Retinol dehydrogenase inhibitor	93.4	0.0
Antieczematic	89.6	0.5
Alpha-pinene-oxide decyclase inhibitor	88.1	0.1
Apoptosis agonist	81.6	0.7
Antimycobacterial	61.0	0.9
Antiviral (Rhinovirus)	57.4	0.9
Antifungal	58.2	2.0
Isoprene		
Aspulvinone dimethylallyltransferase inhibitor	95.0	0.3
Cl−transporting ATPase inhibitor	88.2	0.3
Carminative	86.1	0.3
Antineoplastic	85.8	0.6
Mucomembranous protector	85.9	0.8
Antiviral (Rhinovirus)	65.2	0.4
Antiseborrheic	63.7	5.0
Antifungal	48.7	3.3
Antimycobacterial	35.2	6.0

Table 14. Prediction of biological activity of natural rubber extracts using the PASS-online software package

Activity	P_a, %	P_i, %
Cyclododecane		
Aspulvinone dimethylallyltransferase inhibitor	93.3	0.4
Nicotinic alpha6beta3beta4alpha5 receptor antagonist	92.8	0.2
Testosterone 17beta-dehydrogenase (NADP+) inhibitor	92.5	0.4
Ubiquinol-cytochrome-c reductase inhibitor	92.5	0.4
Glucan endo-1,6-beta-glucosidase inhibitor	92.2	0.3
Antiseborrheic	84.9	1.0
Antiviral (Picornavirus)	68.4	0.7
Antiviral (Adenovirus)	51.6	0.5
Antiviral (Influenza)	48.5	2.5
Antibacterial, ophthalmic	31.3	0.4
Antifungal	30.1	8.0
4,7-dimethyldecane		
Acrocylindropepsin inhibitor	94.3	0.3
Chymosin inhibitor	94.3	0.3
Saccharopepsin inhibitor	94.3	0.3
Testosterone 17beta-dehydrogenase (NADP+) inhibitor	92.6	0.4
5 Hydroxytryptamine release stimulant	92.5	0.4
Polyporopepsin inhibitor	92.4	0.4
Antiseborrheic	85.7	0.9
Antiviral (Picornavirus)	61.8	1.6
Antiviral (Rhinovirus)	55.9	1.1
Antianginal	48.1	4.7
Antifungal	46.6	3.7
Antiviral (Influenza)	45.7	3.1
Antiviral (Herpes)	42.6	2.5

The following types of activity were also predicted for these two components: fungicidal, antiviral and antimycobacterial with probabilities of manifestation from 35.2 to 61% [13].

The prediction of the biological activity of the NR components, listed in Table 14, showed that cyclododecane and 4,7-dimethyldecane are characterized by the manifestation of two types of activity with the highest probabilities (93.3−94.3%) — an inhibitor of aspulvinondimethylallyltransferase and an inhibitor of acrocylindropepsin, respec-

tively. The manifestation of antiviral, fungicidal, antibacterial and other types of activities was also predicted for these substances [24]. It should be noted that inhibition of even one enzyme involved in an important metabolic process suspends the entire process, and sometimes can be fatal for a microorganism.

Conclusion

Acetone extracts of NR and SR of various brands were obtained and their biological activity was determined. It was shown by the ATR IR spectroscopy method that the extracts of the studied rubbers have different compositions and contain different amounts of stearic, oleic and linoleic acids. At the same time, NR extracts contain proteins, enzymes, and sugars that have a significant effect on microbiological activity.

Test cultures of various microorganisms were used for determining the biological activity of rubber extracts: bacteria, fungi and yeast.

Thus, it was shown that the zone of inhibition of yeast it Saccharomyces boulardii CNCM I-745 decreases by 2 times with an increase of the concentration of NR extract from 1 to 50 mg/m*l*. On the contrary, the inhibition zone increases by 2 times in the same concentration range, with an increase of the concentration of the SR extract. The best results with respect to inhibition of yeast growth *Candida tropicalis* were shown by SR extracts with concentrations ranging from 0.1 to 50 mg/m*l*.

With an increase of the concentration of extracts of both NR and SR (in the concentration range from 1 to 30 mg/m*l*), the size of the zone of inhibition of growth of gram-positive spore-forming bacteria it Bacillus subtilis decreases, i.e. the antibacterial properties of the extracts become worse.

It was shown on a test culture of widespread mold fungi *Aspergillus niger* that the extract of synthetic rubber SKI-5 PM has a maximum rate of growth inhibition (1.2 ± 0.1 mm/day) compared with extracts of NR (0.8 ± 0.2 mm/day). At the same time, NR and SR extracts equally exhibit fungicidal activity against colonies of black mold fungi *Alternaria alternata*.

The theoretical calculation of the biological activity of the components of NR and SR predicts different types of activity: antibacterial, fungicidal, antiviral and others.

A comparative screening assessment of the antibacterial and fungicidal properties of the extracts obtained by the criterion of changing the size of the microbial growth inhibition zone showed that synthetic rubbers SKI-5 PM are the most promising products for use in the medical and food industries.

Acknowledgments

The authors would like to thank Ph. D. (Chem.) S.I. Korotkov, R.A. Chebotar (FSBI „NIISK“) and Ph. D. (Eng), Associate Professor E.B. Aronova (IBSiB SPbPU) for technical assistance in conducting microbiological experiments and discussing the results.

Funding

The study was financed by Federal State Budgetary Institution „NIISK“.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] J.M. Martin, W.K. Smith. *Proizvodstvo i primenenie rezinotekhnicheskikh izdelij* (Professiya, CPb., 2006) (in Russian)
[2] TU 2294-016-73776139-2009. *Synthetic cis-isoprene rubber SKI-5 PM.* (in Russian)
[3] A. Rahimi, A. Mashak. Rev. Rubbers in Medicine: Natural, Silicone and Polyurethane Rubbers, **42** (6), 223 (2013). DOI: 10.1179/1743289811Y.0000000063
[4] V.A. Vasiliev, I.S. Nasyrov. *Otechestvennye promyshlennye stereoregulyarnye kauchuki. Issledovaniya i razrabotki* (Bashk. enciklopediya, Ufa, 2018) (in Russian)
[5] B.R. Kuluyev, R.R. Garafutdinov, I.V. Maksimov, A.M. Sagitov, D.A. Chemeris, A.V. Knyazev, Z.R. Vershinina, An.X. Baymiev, A.A. Muldashev, Al.X. Baymiev, A.V. Chemeris. Biomika, **7** (4), 224 (2015) (in Russian).
[6] B.A. Guesskin, A.A. Dontsov, V.A. Shershnev. *Khimiya elastomerov* (Khimiya, M., 1981) (in Russian)
[7] TU 2294-051-16810126-2004. *Rubber SKI-5 PM. TU.* (in Russian)
[8] I.V. Harmonov. *Sinteticheskij kauchuk* (Khimiya, L., 1983) (in Russian)
[9] E.B. Sviridov. *Kniga o polimerakh: svojstva i primenenie, istoriya i segodnyashnij den' materialov na osnove vysokomolekulyarnykh soedinenij* (ESAFU, Arhangelsk, 2016) (in Russian)
[10] GOST ISO 1407-2013. *Rubber. Determination of solvent extractable substances* (Standartinform, M., 2014) (in Russian)
[11] GOST 24919-91. *Rubber. Definition of solvent extract* (Publishing House of Standards, Moscow, 1992) (in Russian)
[12] Methodological guidelines 4.2.1890−04. Determination of the sensitivity of microorganisms to antibacterial drugs. Clinical microbiol. antimicrobial. chemother. **6** (4), 306 (2004) (in Russian).
[13] E.N. Rieger. *Izuchenie antimikrobnykh svojstv ekstraktov kauchukov s ispol'zovaniem test-kul'tur* (Magisterskaya diss., SPbPU, SPb., 2023) (in Russian)
[14] T.S. Kalebina, I.S. Kulaev. Uspekhi biologicheskoy khimii **41**, 105 (2001) (in Russian).
[15] D. Yagnik, V. Serafin, J. Ajit. Shah. Sci. Rep., **8**, 1732 (2018). DOI: 10.1038/s.41598-017-18618-x.
[16] D.G. Zvyagintsev. *Pochva i mikroorganizmy* (Izd-vo MGU, M., 1987) (in Russian)
[17] M.V. Gorlenko. *Vse o gribakh* (Vysshaya shkola, M., 1986) (in Russian)

[18] Electronic resource. Available at: http://bio.niv.ru/doc/encyclopedia/life-of-plants/articles/352/rod-aspergill-aspergillus.htm
[19] K.S. Bolotov. *Klassifikaciya i morfologicheskie osobennosti mikroorganizmov* (SAFU, Arkhangelsk, 2016) (in Russian)
[20] Electronic resource. Available at: https://studopedia.org/14-95608.html
[21] GOST 28665-90 (ISO 4650-84). *is Rubber. Identification. Infra-red spectrometric method* (Publishing House of Standards, Moscow, 1991)
[22] E.I. Knerelman, R.S. Yarullin, G.I. Davydova, G.P. Startseva, V.Ya. Churkina, P.E. Matkovsky, S.M. Aldoshin. Vestnik Kaz. tekh. un-ta, **6**, 68 (2008) (in Russian).
[23] Kh.T. Chan, A.P. Rakhmatullina. Vestnik tekh. un-ta, **20** (8), 69 (2017) (in Russian).
[24] D.A. Filimonov, A.A. Lagunin, T.A. Gloriozova, A.V. Rudik. Chem. Heterocyclic Compounds, **50** (3), 444 (2014). DOI: 10.1007/s10593-014-1496-1

Translated by A.Akhtyamov