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IN VITRO ANTIMICROBIAL ACTIVITY OF BRONCHOSOL

EWA WITKOWSKA-BANASZCZAK[1]*, ANNA MICHALAK[1] and ANNA KĘDZIA[3]

[1] Department of Pharmacognosy, University of Medical Sciences in Poznan,
4 Święcickiego St., 60-781 Poznań, Poland
[2] Phytopharm Klęka SA, Medical Affairs Department, Klęka 1, 63-040 Nowe Miasto nad Wartą, Poland
[c]Department of Oral Microbiology at Chair of Microbiology of Medical University of Gdansk,
38 Do Studzienki St., 80-227 Gdańsk, Poland

Abstract: Bronchosol is a traditional medicinal product in the form of syrup used in cough and impeded expectoration. The active ingredients that it contains include extracts from the herb of thyme, the root of primrose and thymol. It is recommended in disorders of the respiratory tract when expectoration is impeded and secretion of liquid mucus in bronchi is insufficient. Antimicrobial activity of the components of Bronchosol, especially thyme and thymol, has frequently been reported in the literature. To date, there have not been any studies to confirm such activity of Bronchosol, though. The results of our research are the first one to point to the great activity of Bronchosol against microorganisms causing infections of the respiratory tract. It has been demonstrated that this product displayed antimicrobial activity against reference strains as well as strains of anaerobic and aerobic bacteria and fungi isolated from patients. The confirmation of the antimicrobial activity of Bronchosol provides an explanation of its effectiveness in the therapy of the respiratory tract infections.

Keywords: Bronchosol, antibacterial activity, antifungal activity, *Thymi herba, Primulae radix*

A cough is one of the most common symptoms of respiratory infections mostly of viral or/and bacterial etiology (1, 2). The main infectious factors include influenza and parainfluenza viruses, RS-virus, rhinoviruses and adenoviruses (3, 4). *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pyogenes* are primarily responsible for bacterial respiratory infections. They are also, yet less frequently, caused by Gram-negative baccilli, i.e., *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter spp.* and Gram-positive cocci from the *Staphylococcus aureus* species. Various species from the genera *Peptostreptococcus, Micromonas, Finegoldia, Prevotella, Porphyromonas, Fusobacterium, Bacteroides, Propionibacterium, Bifidobacterium, Leptotrichia, Tissierella* and *Selenomonas* are often enumerated among anaerobic bacteria that cause infections of the upper and lower respiratory tract. Sometimes, baccilli from the genus *Legionella* or atypical bacteria, i.e., *Chlamydia pneumoniae* and *Mycoplasma pneumoniae,* are the source of infection. On the other hand, yeast-like fungi, particularly from the genera *Candida albicans*, *Pneumocistis jiroveci* or protozoa, participate in such infections much more seldom (5-9). Great effectiveness and very high tolerance of preparations containing extracts from the herb of thyme and the root of primula have been shown in the treatment of respiratory infections, including acute bronchitis (10-12).

One such preparation is Bronchosol, syrup available on the Polish market since 2002. The active ingredients that it contains are a dense composite extract made of the herb of thyme, primula root and furthermore thymol. The herb of thyme (*Thymi* herba) and the root of primula (*Primulae* radix) are used in infections of the upper respiratory tract as agents that make expectoration of lingering mucus easier and stimulate secretion of liquid mucus in bronchi. The healing activity is connected with the presence of active compounds, mainly saponins, in the primula root and an essential oil found in thyme (8, 9, 13-17). *In vitro* studies have demonstrated that, apart from the activity making expectoration of lingering phlegm easier and anti-

* Corresponding author: e-mail: banaszcz@ump.edu.pl

inflammatory activity (18, 19), the saponins from primula root show immunostimulant activity (8, 13, 15, 19, 20). Moreover, antimicrobial activity of extracts from primula root has been proven (19), but reports on this topic are sporadic. The essential oil as well as extracts from thyme show expectorant activity. It has been demonstrated that extracts from thyme and thyme oil decrease tension of bronchial smooth muscles and have anti-inflammatory activity (19, 21-24). A number of studies have concerned the antimicrobial activity of the extracts and essential oil from thyme. This activity is connected to a large extent with the presence of thymol, the main component of the essential oil (16, 20, 25, 26). It has been proven that perforation of bacterial cell membrane may be the key way of the antimicrobial activity of thymol. The measurement of the cell content outflow resulting from the activity of thymol has been made for bacteria found in the oral cavity, such as *Porphyromonas gingivalis, Selenomonas artemidis, Streptococcus sorbinus*. The decrease in intracellular ATP observed in *S. sobrinus* and *P. gingivalis* as well as the inhibition of ATP generation in *P. gingivalis* have been associated with the leak of the cell content (27). Great antimicrobial activity of the essential oil from the herb of thyme against bacteria causing respiratory infections most frequently, i.e., *Streptococcus pyogenes* (62 strains), *Streptococcus agalactiae* (20 strains), *Streptococcus pneumoniae* (6 strains) *Klebsiella pneumoniae* (6 strains), *Haemophilus influenzae* (25 strains), *Staphylococcus aureus* (5 strains), S*tenotrophomonas maltophilia* (5 strains), obtained from patients suffering from respiratory infections, has been demonstrated. *Haemophilus influenzae* and *Stenotrophomonas maltophilia* have been most susceptible to the activity of the oil from *Thymus vulgaris*, followed by *Streptococcus pneumoniae*. The *Klebsiella pneumoniae* strain has been least sensitive. The MIC and MBC values have been comparable and ranged from 0.025 mL/mL (*K. pneumoniae)* to 0.003125 mL/mL (*S. maltophilia*). The essential oil has shown antimicrobial activity against the *Streptococcus* spp. and *Haemophilus influenza* strains, which are resistant to the activity of numerous antibiotics, yet it has not been effective against the following antibiotic-resistant strains: *S. aureus*, *K. pneumoniae*, *S. maltophilia* (28). However, the results of the study by Tohidpour et al. (29) suggested antimicrobial activity of the *Thymus vulgaris* oil against methicillin-resistant strains of clinical bacteria isolated from wounds, eyeball and trachea, including *Staphylococcus aureus* (MRSA) and the REFERENCE strains of *Bacillus cereus*, *Escherichia coli*, *K. pneumoniae*, *S. aureus* and *S. aureus* MRSA. The essential oil from thyme inhibited the development of the reference strains. The MIC ranged from 0.1-4 v/v % (35). The essential oil and extracts from thyme have also shown antifungal activity (16, 20, 30).

MATERIALS AND METHODS

Material

Syrup called Bronchosol (Phytopharm, Kleka, Poland) available on the Polish market was used in the study. Hundred milliliters of Bronchosol contain the following active ingredients: 4.36 g of a dense extract consisting of (3 : 1; aq.) thyme and primula root (7.6 : 1) and 19.8 g of thymol (concentration for use). The excipients include orange flavor, sugar and purified water.

The antimicrobial activity of Bronchosol was determined on the following 20 strains of anaerobic bacteria isolated from patients with respiratory infections: *Peptostreptococcus anaerobius* (2 strains), *Finegoldia magna* (2 strains), *Micromonas micros* (2 strains), *Actinomyces odontolyticus* (1 strain), *Bifidobacterium breve* (1 strain), *Propionibacterium acnes* (1 strain), *Propionibacterium granulosum* (1 strain), *Prevotella intermedia* (3 strains), *Prevotella levii* (1 strains), *Prevotella loescheii* (1 strain), *Porphyromonas asaccharolytica* (1 strain), *Porphyromonas gingivalis* (1 strain), *Fusobacterium nucleatum* (1 strain), *Fusobacterium necrophorum* (1 strain), *Bacteroides fragilis* (1 strain) and 6 reference strains: *P. anaerobius*, *F. magna*, *P. acnes*, *B. breve*, *F. nucleatum*, *B. fragilis*.

As far as the aerobic bacteria are concerned, 13 strains (one from each species) were isolated from materials obtained from patients. The species included: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Streptococcus anginosus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Acinetobacter baumannii*, *Citrobacter freundii*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens*. There were 7 reference strains, namely *S. aureus*, *S. pneumoniae*, *S. pyogenes*, *H. influenzae*, *Moraxella catarrhalis*, *K. pneumoniae*, *P. aeruginosa*. Five strains of yeast-like fungi isolated from patients were also subjected to the study and these belonged to the following species: *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*. There were 5 reference strains, too.

The materials collected from patients were inoculated on selective and non-selective media

appropriate for the given microorganisms, which were incubated in anaerobic jar or aerobic conditions for the right period of time. The isolated strains of anaerobic (31-34) and aerobic (33-35) bacteria as well as the fungi (36, 37) were identified in accordance with the present principles.

Methods

Antibacterial activity

Determination of minimal bactericidal concentration

In order to determine the minimal bactericidal concentration (MBC) of Bronchosol, 0.1 mL of a suspension of the anaerobic or aerobic bacterial strains, containing 10^6 CFU (colony forming units) / mL was added to 1 mL of the (undiluted) preparation. Next, after 15 and 30 min, 0.1 mL was taken and inoculated into 2 mL of thioglycolate broth (anaerobic bacteria) or Brain Hearth Infusion (BHI) broth (Merck) (aerobic bacteria). The medium inoculated with 0.1 mL of the appropriate bacteria culture was used to control the growth of a given strain. The incubation of the tested and control media was conducted at 37°C for 48 h in anaerobic jars (in anaerobic conditions) for the anaerobic bacteria, and at 37°C for 24 h in aerobic conditions for the aerobic bacteria. A lack of any bacteria growth in the medium after appropriately long incubation proved bactericidal activity (MBC) of the preparation.

Determination of minimal inhibitory concentration

The minimal inhibitory concentration (MIC) of the bacterial strains was determined by the method of

Table 1. Bactericidal activity (MBC - minimal bactericidal concentration) of Bronchosol at concentration recommended by producer against reference strains of anaerobic bacteria and bacteria isolated from patients with respiratory infections.

Anaerobic bacteria	Number of strains tested	Number of strains sensitive to preparation	
		after 15 min	after 30 min
Gram-positive cocci:			
*Peptostreptococcus anaerobius**	2	2	2
*Finegoldia magna**	2	2	2
*Micromonas micros**	2	2	2
Total Gram-positive cocci	**6**	**6**	**6**
Gram-positive baccilli:			
*Actinomyces odontolyticus**	1	1	1
Bifidobacterium breve	1	1	1
*Propionibacterium acnes**	1	0	1
*Propionibacterium granulosum**	1	0	1
Total Gram-positive baccilli	**4**	**2**	**4**
Total Gram-positive anaerobic bacteria	**10**	**8**	**10**
Gram-negative baccilli:			
*Prevotella intermedia**	3	3	3
*Prevotella levii**	1	1	1
*Prevotella loescheii**	1	1	1
*Porphyromonas asaccharolytica**	1	1	1
*Porphyromonas gingivalis**	1	1	1
*Fusobacterium nucleatum**	1	1	1
*Fusobacterium necrophorum**	1	0	1
*Bacteroides fragilis**	1	0	0
Total Gram-negative baccilli	**10**	**8**	**9**
Standard strains			
Finegoldia magna ATCC 29328	1	1	1
Peptostreptococcus anaerobius ATCC 27337	1	1	1
Propionibacterium acnes ATCC 11827	1	1	1
Bifidobacterium breve ATCC 15700	1	1	1
Fusobacterium nucleatum ATCC 25586	1	1	1
Bacteroides fragilis ATCC 25585	1	0	0
Total standard strains	**6**	**5**	**5**
Total anaerobic bacteria	**26**	**21**	**24**

* - anaerobic bacteria strains isolated from patients with respiratory infections, 0 - no effect of the preparation.

serial dilution of Bronchosol on the right medium. In the case of the anaerobic bacteria, Brucella agar with 5% of defibrinated sheep blood, menadione and hemin was used, while Mueller-Hinton agar was used for the aerobic bacteria. The following concentrations of the preparation were tested: 6.2, 12.5, 25.0, 50.0, 100.0 and 200.0 mg/mL. A suspension containing 10^6 CFU per drop was placed on the surface of the right agar (depending on the bacteria type) with Steers replicator, after previous addition of the appropriate concentration of the preparation to the agar. Agar which did not contain Bronchosol was used to control the strain growth. The inoculated media and the control media were incubated in either anaerobic (in anaerobic jars) or aerobic conditions, at 37°C for the right period of time depending on the bacteria type: for 48 h in the case of the anaerobic bacteria and for 24 h in the case of the aerobic bacteria. The MIC was defined as the lowest concentrations of the preparation that completely inhibited the growth of the tested anaerobic or aerobic bacteria strains.

The yeast-like fungi strains cultured from the materials obtained from patients with infections of the upper respiratory tract were inoculated in Sabouraud's agar and incubated at 37°C for 24-72 h.

Antifungal activity

Determination of minimal fungicidal concentration

In order to assess the fungicidal activity (MFC - minimal fungicidal concentration), 0.1 mL of a suspension of the culture of the tested strain containing 10^6 CFU in 1 mL was added to 1 mL of Bronchosol (undiluted preparation). After 15 and 30 min, samples of 0.1 mL were taken and inoculated into 2 mL of BHI broth (Merck). The BHI broth inoculated with 0.1 mL of the fungal culture constituted the growth control of a given strain. The inoculated and control media were incubated at 37°C for 24 h in aerobic conditions. A lack of any yeast-like fungi growth in the medium proved fungicidal activity of the preparation.

Table 2. Bactericidal activity (MBC - minimal bactericidal concentration) of Bronchosol at concentration recommended by producer against reference strains of aerobic bacteria and bacteria isolated from patients with respiratory infections.

Aerobic bacteria	Number of strains tested	Number of strains sensitive to preparation	
		after 15 min	after 30 min
Gram-positive cocci:			
*Staphylococcus aureus**	1	1	1
*Staphylococcus epidermidis**	1	1	1
*Enterococcus faecalis**	1	0	0
*Streptococcus anginosus**	1	1	1
*Streptococcus pneumoniae**	1	1	1
*Streptococcus pyogenes**	1	1	1
Total Gram-positive cocci	**6**	**5**	**5**
Gram-negative baccilli:			
*Acinetobacter baumannii**	1	1	1
*Citrobacter freundii**	1	0	1
*Escherichia coli**	1	1	1
*Haemophilus influenzae**	1	0	1
*Klebsiella pneumoniae**	1	0	1
*Pseudomonas aeruginosa**	1	0	0
*Serratia marcescens**	1	1	1
Total Gram-negative baccilli	**7**	**3**	**6**
Standard strains:			
Staphylococcus aureus ATCC 25923	1	1	1
Streptococcus pneumoniae ATCC 49619	1	1	1
Streptococcus pyogenes ATCC 19615	1	1	1
Haemophilus influenzae ATCC 49274	1	1	1
Moraxella catarrhalis ATCC 25238	1	1	1
Klebsiella pneumoniae ATCC 13883	1	0	1
Pseudomonas aeruginosa ATCC 27853	1	0	0
Total standard strains	**7**	**5**	**6**
Total aerobic bacteria	**20**	**13**	**17**

* - aerobic bacteria strains isolated from patients with respiratory infections, 0 - no effect of the preparation.

Table 3. Sensitivity (MIC - minimal inhibitory concentration) to Bronchosol of anaerobic bacteria isolated from patients with respiratory infections and that of reference strains.

Anaerobic bacteria	Number of strains tested	MIC [mg/mL]					
		≥ 200.0	100.0	50.0	25.0	12.5	6.2
Gram-positive cocci:							
*Peptostreptococcus anaerobius**	2	1	1	0	0	0	0
*Finegoldia magna**	2	1	1	0	0	0	0
*Micromonas micros**	2	1	1	0	0	0	0
Total Gram-positive cocc	**i 6**	**3**	**3**	**0**	**0**	**0**	**0**
Gram-positive baccilli:							
*Actinomyces odontolyticus**	1	0	1	0	0	0	0
*Bifidobacterium breve**	1	0	1	0	0	0	0
*Propionibacterium acnes**	1	1	0	0	0	0	0
*Propionibacterium granulosum**	1	1	0	0	0	0	01
Total Gram-positive baccilli	**4**	**2**	**2**	**0**	**0**	**0**	**0**
Gram-negative baccilli:							
*Prevotella intermedia**	3	3	0	0	0	0	0
*Prevotella levii**	1	1	0	0	0	0	0
*Prevotella loescheii**	1	1	0	0	0	0	0
*Porphyromonas asaccharolytica**	1	1	0	0	0	0	0
*Porphyromonas gingivalis**	1	1	0	0	0	0	0
*Fusobacterium nucleatum**	1	1	0	0	0	0	0
*Fusobacterium necrophorum**	1	1	0	0	0	0	0
*Bacteroides fragilis**	1	1	0	0	0	0	0
Total Gram-negative baccilli	**10**	**10**	**0**	**0**	**0**	**0**	**0**
Standard strains:							
Finegoldia magna ATCC 29328	1	0	1	0	0	0	0
Peptostreptococcua anaerobius ATCC 27337	1	1	0	0	0	0	0
Propionibacterium acnes ATCC 11827	1	1	0	0	0	0	0
Bifidobacterium breve ATCC 25286	1	1	0	0	0	0	0
Fusobacterium nucleatum ATCC 25586	1	1	0	0	0	0	0
Bacteroides fragilis ATCC 25285	1	1	0	0	0	0	0
Total standard strains	**6**	**5**	**1**	**0**	**0**	**0**	**0**
Total anaerobic bacteria	**26**	**20**	**6**	**0**	**0**	**0**	**0**

* - anaerobic bacteria strains isolated from patients with respiratory infections, 0 - no effect of the preparation.

The sensitivity test (MIC) of the yeast-like fungi to Bronchosol was conducted by the method of serial dilution in Sabouraud's agar (38). The following dilutions of the preparation were tested: 6.2, 12.5, 25.0, 50.0, 100.0 mg/mL. An inoculum containing 10^6 CFU per drop was placed on the surface of the agar with Steers replicator. A medium without the preparation was used to control the growth of the tested fungi strains. Incubation of the inoculated and control media was performed at 37^{o}C for 24 h in aerobic conditions. The MIC was defined as the lowest concentrations of Bronchosol which completely inhibited the growth of the tested strains of yeast-like fungi.

RESULTS AND DISCUSSION

The aim of the study was to determine the antimicrobial activity of Bronchosol (Phytopharm, Klęka) against anaerobic and aerobic bacteria, and fungi that cause respiratory infections most frequently, isolated from patients with respiratory infections, and reference ones.

The study was conducted in *in vitro* conditions slightly different from *in vivo* conditions. All tests were performed at a temperature of 37^{o}C, similar to the temperature found in the oral cavity and upper respiratory tract of a healthy organism. A bacterial, viral or fungal infection usually causes a rise in the temperature, which can be controlled with the use of antipyretics. The neutral pH found in the oral cavity and upper respiratory tract is similar to the pH reaction of the applied media (6.4 – 7.4), except for Sabouraud's agar – pH 5.4, whose acidic reaction corresponds to pH of the oral cavity during and after a meal. The exposure of the microorganisms to Bronchosol in the experimental conditions lasted 48

Table 4. Sensitivity (MIC - minimal inhibitory concentration) to Bronchosol of aerobic bacteria isolated from patients with respiratory infections and that of reference strains.

Aerobic bacteria	Number of strains tested	MIC [mg/mL]					
		≥ 200.0	100.0	50.0	25.0	12.5	6.2
Gram-positive cocci:							
*Staphylococcus aureus**	1	0	0	1	0	0	0
*Staphylococcus epidermidis**	1	0	0	0	0	0	1
*Enterococcus faecalis**	1	1	0	0	0	0	0
*Streptococcus anginosus**	1	0	1	0	0	0	0
*Streptococcus pneumoniae**	1	0	0	1	0	0	0
*Streptococcus pyogenes**	1	0	0	0	0	1	0
Total Gram-positive cocci	**6**	**1**	**1**	**2**	**0**	**1**	**1**
Gram-negative baccilli:							
Acinetobacter baumannii *	1	0	1	0	0	0	0
*Citrobacter freundii**	1	1	0	0	0	0	0
*Escherichia coli**	1	1	0	0	0	0	0
*Haemophilus influenzae**	1	0	0	1	0	0	0
*Klebsiella pneumoniae**	1	1	0	0	0	0	0
*Pseudomonas aeruginosa**	1	1	0	0	0	0	0
*Serratia marcescens**	1	1	0	0	0	0	0
Total Gram-negative baccilli	**7**	**5**	**1**	**1**	**0**	**0**	**0**
Standard strains:							
Staphylococcus aureus ATCC 25923	1	0	1	0	0	0	0
Streptococcus pneumoniae ATCC 49619	1	1	0	0	0	0	0
Streptococcus pyogenes ATCC 19615	1	0	0	0	0	1	0
Haemophilus influenzae ATCC 49247	1	0	0	1	0	0	0
Moraxella catarrhalis ATCC 25238	1	1	0	0	0	0	0
Klebsiella pneumoniae ATCC 13883	1	1	0	0	0	0	0
Pseudomonas aeruginosa ATCC 27853	1	1	0	0	0	0	0
Total standard strains	**7**	**4**	**1**	**1**	**0**	**1**	**0**
Total aerobic bacteria	**20**	**10**	**3**	**4**	**0**	**2**	**1**

* - aerobic bacteria strains isolated from patients with respiratory infections, 0 - no effect of the preparation.

Table 5. Fungicidal activity (MFC - minimal fungicidal concentration) of Bronchosol at concentration recommended by producer against reference strains of yeast-like fungi and strains isolated from patients with respiratory infections.

Yeast-like fungi	Number of strains tested	Number of strains sensitive to preparation	
		after 15 min	after 30 min
*Candida albicans**	3	3	3
*Gandida glabrata**	1	1	1
*Candida krusei**	1	1	1
*Candida parapsilosis**	1	1	1
*Candida tropicalis**	1	1	1
Total yeast-like fungi	**7**	**7**	**7**
Standard strains:			
Candida albicans ATCC 10231	1	1	1
Candida glabrata ATCC 66032	1	1	1
Candida krusei ATCC 14234	1	1	1
Candida parapsilosis ATCC 22019	1	1	1
Candida tropicalis ATCC 750	1	1	1
Total standard strains	**5**	**5**	**5**
Total yeast-like fungi	**12**	**12**	**12**

* - yeast-like fungi strains isolated from patients with respiratory infections.

Table 6. Sensitivity (MIC - minimal inhibitory concentration) to Bronchosol of yeast-like fungi from genus *Candida* isolated from patients with respiratory infections and that of reference strains.

Yeast-like fungi	Number of strains tested	MIC [mg/mL]					
		≥ 200.0	100.0	50.0	25.0	12.5	6.2
*Candida albicans**	3	1	1	1	0	0	0
*Candida glabrata**	1	0	1	0	0	0	0
*Candida krusei**	1	1	0	0	0	0	0
*Candida parapsilisis**	1	0	1	0	0	0	0
*Candida tropicalis**	1	0	1	0	0	0	0
Total yeast-like fungi	**7**	**2**	**4**	**1**	**0**	**0**	**0**
Standard strains:							
Candida albicans ATCC 10231	1	0	1	0	0	0	0
Candida glabrata ATCC 66032	1	1	0	0	0	0	0
Candida krusei ATCC 14234	1	1	0	0	0	0	0
Candida parapsilosis ATCC 22019	1	0	1	0	0	0	0
Candida tropicalis ATCC 750	1	0	1	0	0	0	0
Total standard strains	**5**	**2**	**3**	**0**	**0**	**0**	**0**
Total yeast-like fungi	**12**	**4**	**7**	**1**	**0**	**0**	**0**

* - yeast-like fungi strains isolated from patients with respiratory infections, 0 - no effect of the preparation.

and 24 h in the case of the anaerobic and aerobic bacteria, respectively, and between 24 and 72 h for the fungi. In *in vivo* conditions, the exposure of the microorganisms to the ingredients of the preparation was initially limited to several-minute local activity of the syrup, which covers the mucosa of the oral cavity and throat; however, after absorption of the active ingredients by the mucosa of the oral cavity and throat as well as from the digestive tract, the active ingredients get with blood to the lower and upper respiratory tract, where they show antimicrobial activity.

The majority of the strains used in the study were isolated from material obtained from patients with respiratory infections; 13 of them were reference strains.

The preparation showed great bactericidal activity (concentration for use) against all 46 bacterial strains tested. In the case of the anaerobic bacteria, 15 min after application of Bronchosol, 80% of the Gram-positive bacteria were killed, including 60% of the cocci, and after 30 min all the tested strains of these bacteria were killed. Similar bactericidal activity was observed against the Gram-negative anaerobic baccilli, 80% of which were killed after 15 min and 90% after 30 min after application of Bronchosol. It is worth pointing out that after 15 min, the preparation showed bactericidal activity against 80% of all the anaerobic bacteria tested, and after 30 min – against 95% of these bacteria (Table 1). In the case of the aerobic bacteria, Bronchosol showed great activity, only 15 min after application, against the Gram-positive cocci (83%), including the bacteria strains which participate in respiratory infections more frequently, i.e., *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Streptococcus anginosus*. This activity did not change after 30 min since the application of the preparation. The Gram-negative baccilli, however, were less susceptible to the activity of Bronchosol. After 15 min of its activity, 43% of the strains were killed, but the biocidal activity increased 30 min after the application of the preparation and affected 86% of the strains, including those from the following species: *Acinetobacter baumannii*, *Escherichia coli*, *Haemophilus influenzae* and *Klebsiella pneumonia* (Table 2).

The results of the study in sensitivity (MIC) (Table 3) suggested that Bronchosol ranging between ≤ 6.2 – 100.0 mg/mL inhibited growth of the Gram-positive anaerobic bacteria, including half of the investigated cocci. The activity against the Gram-negative baccilli was lower (MIC ≥ 200.0 mg/mL). The aerobic bacteria tested, on the other hand, demonstrated greater sensitivity to the preparation in question (Table 4). Growth of the Gram-positive cocci was inhibited when concentration ranged between ≤ 6.2 – 100.0 mg/mL (83%). The greatest sensitivity was shown, among others, by strains from the genus *Streptococcus pyogenes*, which often participate in infections of the respiratory tract (MIC ≤ 6.2 – 12.5 mg/mL). The strains of the Gram-negative baccilli turned out to be less sensitive. Out of the 7 tested strains, only 2 (29%) showed sensitivity when the concentration ranged

from 50.0 to 100.0 mg/mL. Growth of the other strains was inhibited when concentration was ≥ 200.0 mg/mL. The greatest sensitivity among the Gram-negative baccilli was displayed by strains from the species *Acinetobacter baumannii* and *Haemophilus influenzae* (MIC ranging from 50.0 to 100.0 mg/mL). High fungicidal activity of Bronchosol against all the tested fungi from the genus *Candida* was also observed. All the investigated strains were killed 15 min after application of the preparation (Table 5). Five (71%) strains of the tested yeast-like fungi from the genera *Candida* were sensitive (MIC) to concentration ranging from 50.0 to 100.0 mg/mL. To inhibit growth of the other strains (29%), higher concentration of Bronchosol, more than 200.0 mg/mL, was required (Table 6).

The strong activity against the abovementioned pathogens probably resulted from synergism between the compounds of thyme, especially the components of the essential oil, primula root and thymol. It should be emphasised that some of the bacteria and fungi were highly sensitive to Bronchosol and, therefore, the antibacterial and antifungal effects were achieved with concentrations several times lower than the ones usually applied. The bacterial strains, frequently participating in infections and demonstrating resistance to medicines, i.e., for example, *Staphylococcus aureus* or the fungi *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, turned out to be sensitive to the activity of Bronchosol.

CONCLUSION

Application of multicomponent preparations enables to obtain a stronger desired effect at lower concentrations of substances and extracts, and decreases the risk of drug resistance of bacteria. The activity against fungi from the genus *Candida* is especially beneficial in the treatment of respiratory infections. Antibiotic therapy leads to destruction of physiological bacterial flora and occurrence of the right conditions for the development of candidosis. The antifungal activity of Bronchosol may prevent candidosis in patients treated with antibiotics.

Thus, Bronchosol is a syrup recommended not only to treat the symptoms of respiratory infections (a cough, impeded expectoration of lingering phlegm), but, more importantly in light of the aforementioned results, it may also be effective as a basic/primary drug affecting the cause of the illness – bacteria and fungi, and as a supporting preparation in an antibiotic therapy.

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