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ANTIMICROBIAL ACTIVITY OF PREPARATION BIOARON C®

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Abstract: The antimicrobial activity of sirupus Bioaron C®, a preparation, whose main ingredient is an extract from the leaves of *Aloe arborescens,* was tested against different microorganisms isolated from patients with upper respiratory tract infections. The experiments were performed on 40 strains: 20 strains of anaerobic bacteria, 13 strains of aerobic bacteria and 7 strains of yeast-like fungi from the genus *Candida* and on 18 reference strains (ATCC). The antimicrobial activity of Bioaron C (MBC and MFC) was determined at undiluted concentration. Bioaron C proved to be very effective against the microorganisms causing infections. At the concentration recommended by the producer, the preparation showed biocidal activity (MBC, MFC) against the strains of the pathogenic microorganisms, which cause respiratory infections most frequently, including, among others, *Peptostreptococcus anaerobius, Parvimonas micra, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus anginosus, Haemophilus influenzae, Moraxella catarrhalis, Pseudomonas aeruginosa* and *Candida albicans*, already after 15 min. The MIC of Bioaron C against most of the tested microorganisms was 5 to 100 times lower than the usually applied concentration. The great antimicrobial activity means that the preparation may be used in the prevention and treatment of infections of the upper respiratory tract. Bioaron C® may be an alternative or complement to classical therapy, especially in children.

Keywords: antimicrobial activity, Bioaron C®, *Aloe arborescens*

Infections of the upper respiratory tract are quite frequent, especially in children. Susceptibility to the infections does not only depend on the infectious agents (viruses, bacteria), but also on the system's immunity. The human immune system, similarly to the respiratory system, reaches maturity at the age of 10–12 years. The most common primary cause of the upper respiratory tract catarrhs are viruses, which damage the epithelium of the respiratory tract, thus making it easier for bacterial pathogens to enter. Inflammation is a process during which the cells of the immune system, i.e., monocytes (macrophages), B, T, NK and cytotoxic lymphocytes and granulocytes are activated to protect the body against harmful stimuli. Treatment of a cold should, then, consist in alleviating the ailments and strengthening the immune system. A hasty use of antibiotic therapy damages the natural bacterial flora, impairing the functioning of the immune system, and often contributes to bacterial resistance to antibiotics, which, in consequence, leads to infection relapses. In addition, upper respiratory tract catarrh in children is often accompanied by appetite disorders, chronic diarrhea, oral candidiasis, or even inhibition of weight and height gain (1–5). Therefore, it seems necessary to search for ways of improving immunity, especially in individuals with its deficiencies, i.e., children susceptible to infections, elderly people or ones with chronic diseases and children who suffered from infections.

There are not many medicines on the Polish pharmaceutical market whose usefulness in the prophylaxis of chronic respiratory infections has been documented. For over 20 years, syrup Bioaron C®, a preparation registered in Poland as a medicinal product, whose main ingredient is an extract from the leaves of candelabra aloe (*Aloe arborescens* Mill.), has been effectively used to prevent such infections. Moreover, Bioaron C® contains vitamin C and thickened juice from the fruit of black choke-

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berry (*Aronia melanocarpa* Elliot.). The ingredients of the preparation show synergism in the immunostimulant activity, contributing to an improvement in the body's resistance to infections, particularly those within the respiratory tract (2, 5–9). Furthermore, they inhibit infection development by modulating transcription of the enzymes participating in chronic inflammations (10). The preparation is intended for children in upper respiratory tract infections and lack of appetite in the course of or after prolonged illness. It is available in pharmacies in Poland, Russia and the Ukraine (4).

The main ingredient of Bioaron C® is an aqueous extract from the leaves of *Aloe arborescens* – one of about 400 species of aloe, from the family Asphodelaceae, which, being tropical plants, are endemic to southern and eastern Africa. The medicinal and cosmetic properties of aloe were already known in ancient Egypt. Nowadays, aloe is grown in greenhouses in many countries. In Poland, it is cultivated for medicinal purposes by Phytopharm Kleka SA only, where leaves of candelabra aloe are obtained from 3-year-old plants for production of medicines such as Bioaron C®. Fresh *Aloe arborescens* leaves are used to make juice or aqueous extracts, which, thanks to the content of numerous active compounds, are used to improve immunity, but also show antimicrobial activity. From the pharmacological viewpoint, the most important components of candelabra aloe are carbohydrates, both polysaccharides (up to 30% of mucilage) and monosaccharides, including glucans, mannans, glucomannans and galactourans. Another group of compounds are glycoproteins (aloectin A and B). Moreover, aloenins – glycoside derivatives of α-pirone, sterols (campesterol, ß-sitosterol), organic acids (salicylic, citric, malic and veratric acid), amino acids, enzymes, vitamins, microelements and a small concentration of aloin are present (4, 11–14). Numerous *in vitro* and *in vivo* studies have confirmed the immunostimulant, antiviral and antibacterial activity of the components found in *Aloe arborescens*. Strong immunomodulatory activity of low-mass polysaccharides (up to 400 KDa) isolated from aloe gel, which increase production of cytokines, nitrogen oxide release and expression of surface and phagocyte particles has been shown on mouse macrophages in *in vitro* studies. The same polysaccharides have also demonstrated anticancer activity *in vivo* (11). *In vitro* studies have confirmed immunomodulatory and hemagglutinating properties of some glycoproteins, which exhibit anti-inflammatory activity, too (12, 15). The antiviral activity of candelabra aloe proven in many *in vitro* studies results from the influence on replication of the human rhinovirus type 14 (5). The immunomodulatory activity, in turn, consists in normalization of T lymphocytes level and a decrease in the activity of proinflammatory cytokines. The leaf extract stimulates synthesis of the interferon responsible for the inhibition of virus replication, raises the level of antibodies in the organism and stimulates tissue regeneration processes. The results of clinical studies suggest that children over 3 years old who have taken 5 mL of an aloe extract twice a day for at least 3 weeks have suffered from upper respiratory tract infections much more seldom (1, 4). Candelabra aloe extracts also regulate digestive tract functions, protect the liver, lower blood glucose levels, accelerate wound healing, and even show anticancer activity (15–18). It has been shown, too, that *Aloe arborescens* extracts exhibit antimicrobial activity (17, 19–20).

Additionally, vitamin C present in Bioaron C® has strong antioxidant activity and contributes to regeneration of the organism. Anthocyanins from chokeberry demonstrate anti-free radical and detoxification activity. It has also been concluded that the ingredients of Bioaraon C promote appetite enhancement, which is particularly important in the case of pediatric and geriatric patients being weakened as a result of an ongoing infection. The preparation is well-tolerated by children (1, 4, 7).

The aim of this study was to evaluate the antimicrobial activity of Bioaron C® against anaerobic and aerobic bacteria as well as yeast-like fungi isolated from patients with upper respiratory tract infections.

MATERIALS AND METHODS

Material for the study

Material for the study was Bioaron C® (Phytopharm, Kleka). Concentration recommended by the producer (undiluted preparation) was used to evaluate the bactericidal (MBC) and fungicidal (MFC) activity. The sensitivity of the microorganisms tested (MIC) to Bioaron C® was determined by the serial dilution method, with the use of the following concentrations of the preparation on media appropriate for a given microorganism: 6.2; 12.5; 25.0; 50.0; 100.0 and 200.0 mg/mL.

Microorganisms

Strains of anaerobic and aerobic bacteria and yeast-like fungi cultured from material collected from patients with upper respiratory tract infections were examined. In addition, strains of reference

microorganisms from the microbiological collection ATCC (American Type Culture Collection) were used in the experiments.

Anaerobic bacteria

The materials collected from patients with upper respiratory tract infections were delivered to the laboratory in containers with transport liquid prepared by the PRAS method. Next, the samples were inoculated into media appropriate for anaerobic bacteria culture, including enriched and selective ones (21). Incubations was performed at 37°C for 14 days in anaerobic jar containing 10% of $C0_2$, 10% of H_2 and 80% of N_2 a palladium catalyst and anaerobiosis indicator. The anaerobic microorganisms cultured were identified in accordance with the present principles, on the basis of their morphological, physiological and biochemical properties, including API 20A tests (bioMerieux), their ability to produce C_1 to C_6 fatty acids and succinic, fumaric and lactic acids from glucose by the gas chromatography method, as well as the colony's UV natural fluorescence ability (22, 23). Twenty strains of anaerobic bacteria from the following genera were used in the study: *Peptostreptococcus* (2 strains), *Finegoldia* (2), *Parvimonas* (2), *Actinomyces* (1), *Bifidobacterium* (1), *Propionibacterium* (2), *Prevotella* (5), *Porphyromonas* (2), *Fusobacterium* (2) and *Bacteroides* (1) as well as 6 reference strains (Table 1).

Aerobic bacteria

The materials from patients were inoculated into enriched and selective media and incubated at 37°C for 24–48 h. Next, the cultured strains were

Table 1. The MBC of Bioaron C® against anaerobic bacteria at concentration recommended by producer and MIC of these bacteria.

Anaerobic bacteria	Number of strains	MBC of Bioaron C®		MIC (mg/mL)
		after 15 min	after 30 min	
Gram-positive cocci				
Peptostreptococcus anaerobius	2	1	2	≥ 200/100*
Finegoldia magna	2	1	2	≥ 200/100*
Parvimonas micra	2	1	2	≥ 200/100*
Gram-positive baccilli				
Actinomyces odontolyticus	1	1	1	≥ 200
Bifidobacterium breve	1	1	1	≥ 200
Propionibacterium acnes	1	0	0	≥ 200
Propionibacterium granulosum	1	0	1	≥ 200
Total Gram-positive anaerobic bacteria	10	5	9	
Gram-negative baccilli				
Prevotella intermedia	3	0	2	≥ 200
Prevotella levii	1	1	1	≥ 200
Prevotella loescheii	1	0	0	≥ 200
Porphyromonas asaccharolytica	1	0	1	≥ 200
Porohyromonas gingivalis	1	0	0	≥ 200
Fusobacterium nucleatum	1	0	0	≥ 200
Fusobacterium necrophorum	1	0	0	≥ 200
Bacteroides fragilis	1	0	0	≥ 200
Total Gram-negative baccilli	10	1	4	
TOTAL ANAEROBIC BACTERIA	20	6	13	
Reference strains				
Finegoldia magna ATCC 29328	1	1	1	≥ 200
Peptostreptococcus anaerobius ATCC 27337	1	0	0	≥ 200
Propionibacterium acnes ATCC 11827	1	0	0	≥ 200
Bifidobacterium breve ATCC 15700	1	1	1	≥ 200
Fusobacterium nucleatum ATCC 25586	1	0	0	≥ 200
Bacteroides fragilis ATCC 25585	1	0	0	≥ 200

0 = no activity of the preparation
≥ 200/100* = ≥ 200 (1 strain) and 100 (1 strain)

identified (with the use of appropriate tests, such as bioMerieux) in accordance with principles being now in force for these bacteria (22–24). The experiments were performed on 13 isolated strains of aerobic bacteria from the following genera: *Staphylococcus* (2 strains), *Enterococcus* (1), *Streptococcus* (3), *Acinetobacter* (1), *Citrobacter* (1), *Escherichia* (1), *Haemophilus* (1), *Klebsiella* (1), *Pseudomonas* (1), *Serratia* (1) and on 7 reference strains (Table 2).

Yeast-like fungi

The materials obtained from patients with upper respiratory tract infections were inoculated into Sabouraud medium and incubated at 37°C for 24–72 h. The strains of the yeast-like fungi cultured were identified on the basis of cell morphology in preparations stained by Gram's method, appearance of the colony on Sabouraud medium and CHROMagar Candida medium (BioRad), the germ tube test, the ability to produce chlamydospores and their biochemical features (20C AUX bioMerieux) (25). Seven strains of yeast-like fungi from the genus *Candida* isolated from infections and 5 reference strains were subjected to the study (Table 3).

Determination of antimicrobial activity

Bactericidal activity (MBC – minimal bactericidal concentration) of Bioaron C® was determined in relation to anaerobic and aerobic bacteria. Suspensions of bacterial strains containing 10^6 CFU (colony forming units) in 1 mL were added to 1 mL of undiluted Bioaron C®; then, after 15 and 30 min, 0.1 mL was taken and inoculated into 2 mL of appropriate broth. In the case of anaerobic bacteria strains, thioglycolate broth was used, while for aerobic bacteria strains BHI broth (Merck) was employed. A medium inoculated with 0.1 mL of the bacteria culture constituted a growth control of the tested strain. Incubation of the inoculations and con-

Table 2. The MBC of Bioaron C® against aerobic bacteria at concentration recommended by producer and MIC of these bacteria.

Anaerobic bacteria	Number of strains	MBC of Bioaron C®		MIC (mg/mL)
		after 15 min	after 30 min	
Gram-positive cocci				
Staphylococcus aureus	1	1	1	≥ 200
Staphylococcus epidermidis	1	1	1	100
Enterococcus faecalis	1	0	1	≥ 200
Streptococcus anginosus	1	0	1	100
Streptococcus pneumoniae	1	1	1	6.2
Streptococcus pyogenes	1	1	1	6.2
Total Gram-positive cocci	6	4	6	
Gram-negative baccilli				
Acinetobacter baumannii	1	0	1	≥ 200
Citrobacter freundii	1	0	1	≥ 200
Escherichia coli	1	1	1	100
Haemophilus influenzae	1	1	1	6.2
Klebsiella pneumoniae	1	1	1	≥ 200
Pseudomonas aeruginosa	1	1	1	≥ 200
Serratia marcescens	1	1	1	100
Total Gram-negative baccilli	7	5	7	
TOTAL ANAEROBIC BACTERIA	13	9	13	
Reference strains				
Staphylococcus aureus ATCC 25923	1	1	1	≥ 200
Streptococcus pneumoniae ATCC 49619	1	1	1	12.5
Streptococcus pyogenes ATCC 19615	1	1	1	6.2
Haemophilus influenzae ATCC 49274	1	1	1	12,5
Moraxella catarrhalis ATCC 25238	1	1	1	50
Klebsiella pneumoniae ATCC 13883	1	1	1	≥ 200
Pseudomonas aeruginosa ATCC 27853	1	1	1	≥ 200

0 = no activity of the preparation

Table 3. The MFC of Bioaron C® against yeast-like fungi at concentration recommended by producer and MIC of these microorganisms.

Yeast-like fungi Number	Number of strains	MFC of Bioaron C®		MIC (mg/mL)
		after 15 min	after 30 min	
Candida albicans	3	1	2	200/100*
Candida glabrata	1	0	0	≥ 200
Candida krusei	1	1	1	≥ 200
Candida parapsilosis	1	1	1	50
Candida tropicalis	1	1	1	200
TOTAL YEAST-LIKE FUNGI	7	4	5	
Reference strains				
Candida albicans ATCC 10231	1	1	1	100
Candida glabrata ATCC 66032	1	0	0	≥ 200
Candida krusei ATCC 14234	1	1	1	≥ 200
Candida parapsilosis ATCC 22019	1	1	1	100
Candida tropicalis ATCC 750	1	1	1	200

0 = no activity of the preparation; 200/100* = 200 (1 strain) and 100 (2 strains)

trol media was performed in anaerobic conditions at 37°C for 48 h (anaerobic strains) or in aerobic conditions at 37°C for 24 h (aerobic bacteria). A lack of any growth of the tested bacteria in an appropriate medium proved MBC of the preparation against the examined strain.

Fungicidal activity (MFC – minimal fungicidal concentration) was evaluated against strains of yeast-like fungi at concentration of Bioaron C® recommended by its producer. The determination of MFC was conducted in the same way as that of MBC in relation to aerobic bacteria – with the use of BHI broth. A lack of any growth of the yeast-like fungi suggested fungicidal (MFC) activity of the preparation.

Sensitivity of the examined microorganisms (MIC – minimal inhibitory concentration) to Bioaron C® was determined by the serial dilution method.

In the case of the MIC study for anaerobic bacteria, the preparation in question was diluted in Brucella agar, supplemented with 5% of defibrinated sheep blood, menadione and hemin (26). In order to test the sensitivity of aerobic bacteria, Bioaron C® was diluted in Mueller-Hinton agar (27), while the determination of MIC for yeast-like fungi was carried out on the preparation diluted in Sabouraud agar (28). Inoculum containing 10^6 CFU per drop was seeded upon the surface of the right medium. A medium without Bioaron C® was microorganisms growth control. Incubation of the media was conducted at 37°C for 48 h in anaerobic jars in anaerobic conditions (for anaerobes) or at 37°C for 24 h in aerobic conditions (for aerobes and fungi). The MIC was assumed to be such dilution of the preparation, which totally inhibited the growth of the tested strains of the investigated microorganisms.

RESULTS

The antimicrobial activity (MBC, MFC and MIC) of Bioaron C® was tested on 40 strains of microorganisms isolated from patients with upper respiratory tract infections. Twenty strains of isolated anaerobic bacteria, Gram-positive – 6 cocci and 4 rods, and 10 Gram-negative rods were used for the study. The next 13 isolated strains were aerobic bacteria (6 Gram-positive cocci and 7 Gram-negative rods). Apart from that, 7 strains of cultured yeast-like fungi from the genus *Candida* were used. Reference strains (6 anaerobes, 7 aerobes and 5 yeast-like fungi) were used as controls in each test. Tables 1–3 present the results.

Activity of Bioaron C® against anaerobic bacteria

Table 1 shows the results of the bactericidal activity (MBC) of Bioaron C® against the anaerobic bacteria isolated from patients with respiratory tract infections at the concentration recommended by the producer and against the reference strains. The preparation exhibited greater activity against Gram-positive anaerobic bacteria in comparison with Gram-negative bacteria. Fifteen min after application, half of the Gram-positive strains were killed, and 30 min after application, as many as 90% of these microorganisms were killed. In the case of Gram-negative anaerobic bacteria, the biocidal activity 15 min after application affected only 1

strain (10%), and 30 min after application – 4 strains of anaerobic bacteria (40%). All in all, 15 min after its application, Bioaron C® demonstrated biocidal activity against 6 out of 20 strains of anaerobic bacteria: *Peptostreptococcus anaerobius*, *Finegoldia magna, Parvimonas micra, Actinomyces odontolyticus, Bifidobacterium breve* and *Prevotella levii* (30%), and 30 min after application, it affected 13 out of 20 tested strains (61%).

Table 1 gives also the results of the investigation into the sensitivity (MIC) of the same strains of anaerobic microorganisms to Bioaron C®. Out of all the isolated anaerobic bacteria, only 3 (50%) of Gram-positive cocci: *Peptostreptococcus anaerobius*, *Finegoldia magna* and *Parvimonas micra* were sensitive to the preparation at concentration equal to 100.0 mg/mL. The other Gram-positive cocci, Gram-positive rods and Gram-negative anaerobic baccilli required concentration of 200.0 mg/mL or more for their growth to be inhibited.

Activity of Bioaron C® against aerobic bacteria

The results of the biocidal activity of Bioaron C® against the aerobic bacteria isolated from infections and the reference strains have been gathered in Table 2. The preparation was effective against Gram-positive aerobic cocci at the concentration recommended by its producer. It killed 4 out of 6: *Staphylococcus aureus* and *S. epidermidis*, *Streptococcus pneumoniae* and *S. pyogenes* (67%) strains as quickly as after 15 min, while after 30 min, all (100%) of the tested Gram-positive aerobic cocci were destroyed. The strains included: *Staphylococcus aureus*, *Enterococcus faecalis, Streptococcus anginosus, Streptococcus pneumoniae* and *Streptococcus pyogenes*, which often cause infections of the upper respiratory tract. Bioaron C® also showed great activity against Gram-negative aerobic rods. It exhibited biocidal activity against 5 strains (71%) already after 15 min, and against all the 7 Gram-negative aerobic baccilli tested after 30 min.

Table 2 contains also the results of the study on the sensitivity of the aerobic bacteria to Bioaron C®. Three out of the 13 tested bacteria (23%) were sensitive to very low concentration (MIC = 6.2 mg/mL), while 4 strains (31%) were sensitive to concentration equal to 100.0 mg/mL. Seven out of the 13 investigated strains (54%) turned out to be sensitive to concentration ranging from = 6.2 to 100.0 mg/mL. This means that more than half of the examined strains appeared to be sensitive to concentration of the preparation 10 to 100 times lower than the usually applied concentration. The rest of the tested aerobic bacteria required greater concentration of the preparation, i.e., = 200.0 mg/mL, for their growth to be inhibited.

Activity of Bioaron C® against yeast-like fungi

Table 3 presents the results of the fungicidal activity (MFC) of Bioaron C® against the yeast-like fungi from the genus *Candida*, isolated from patients as well as the reference strains. It was shown that after 15 min the preparation exhibited fungicidal activity against 4 out of 7 strains: *Candida albicans*, *C. krusei*, C. *parapsilosis* and *C. tropicalis* (57%), and after 30 min – against 5 of the tested fungi (71%) isolated from patients, including the genera *Candida krusei* and *Candida tropicalis*, which are highly resistant to antifungal medicines.

The results of the sensitivity (Table 3), of the same 7 (and 5 reference) strains to Bioaron C® have been gathered. Five (71%) of the strains isolated from patients were sensitive to the preparation when its concentration ranged from 50.0 to 200.0 mg/mL. The other 3 needed greater concentration (MIC > 200.0 mg/mL) for their growth to be inhibited. The concentration inhibiting growth of the fungal strains was 5 to 15 times lower than the concentration used in practice.

DISCUSSION

The results obtained from the study unambiguously suggest that, at the concentration recommended by the producer, Bioaron C® clearly showed biocidal activity against microorganisms isolated from patients with upper respiratory tract infections and analogous reference strains. The study demonstrated that the preparation was effective against half of the evaluated Gram-positive anaerobic bacteria and 10% of the Gram-negative anaerobic bacteria, as well as against 67% of the aerobic bacterial strains and 57% of the yeast-like fungi strains as quickly as after 15 min. Bioaron C® also demonstrated high MIC in relation to the tested microorganisms.

Bioaron C® is a preparation recommended not only prophylactically, but also in the treatment of upper respiratory tract infections. Numerous earlier studies of this preparation confirmed its effectiveness in such infections. Thanks to its ingredients, especially the aqueous extract from candelabra aloe, Bioaron C® is classed as a plant immunomodulator. The improvement of the body's immunity is also influenced by vitamin C and chokeberry juice present in the preparation (1, 2, 6–9, 13, 17).

The present research has confirmed the advisibility of the use of this preparation in upper respira-

tory tract infections caused by bacteria and yeast-like fungi, as well. Due to increased resistance to antibiotics and easy spread of the antibiotic-resistant bacterial strains, for example, *Staphylococcus aureus* or *Klebsiella pneumonia*, proving the activity of Bioaron C® against the strains of the above-mentioned bacteria, among others, offers a possibility to use the preparation in both viral and bacterial infections. Data from the literature on the subject confirm the antibacterial and antifungal activity of candelabra aloe (14, 18–20, 29–32).

The study showed that, out of the 40 strains isolated from patients with upper respiratory tract infections, 19 were killed 15 min after Bioaron C® application at the concentration recommended by its producer, whereas 31 were killed after half an hour. The activity (MIC) of Bioaron C® against most of the tested microorganisms was 5 to 100 times lower than the concentration used in practice. The preparation exhibited high activity against the bacteria, which cause respiratory infections most often, including *Peptostreptococcus anaerobius, Parvimonas micra, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus anginosus, Klebsiella pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Pseudomonas aeruginosa* and *Candida albicans*. Pellizzoni et al. (20) had also proven the bactericidal activity of the fractions from *Aloe arborescens* leaves against, among others, *Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli*. The antibacterial activity of candelabra aloe against *E. coli* was also described by Bisi-Johnson et al. (30) and Coopoosamy and Naido (31). Studies of extracts from the leaves of several kinds of aloe, including *A. arborescens*, demonstrated activity against *E. coli*, *K. pneumoniae* and *C. albicans* (19), while Ghuman i Coopoosamy (17) confirmed high effectiveness of standardized extracts from candelabra aloe against five Gram-positive bacteria, for instance, *Staphylococcus aureus* and *S. epidermidis*, and four Gram-negative bacteria, including *E. coli* rods.

This research into Bioaron C®, similarly to reports in the literature, hereby proves that the preparation shows antimicrobial activity, probably thanks to the extract from aloe contained in it. The great antimicrobial activity allows it to be applied prophylactically as well as in the treatment of upper respiratory tract infections. Bioaron C® may be recommended especially in autumn and winter, or even be an alternative to hasty prescription of antibiotics in some cases. It may also be a valuable complement to classical therapy, particularly in pediatric medical care.

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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