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Curasept pastes and gels as new killers against pathogenic strains from green and purple complexes present in the oral cavity

Pasty i żele Curasept jako nowi zabójcy szczepów patogennych z kompleksów zielonych i niebieskich obecnych w jamie ustnej

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Keywords

MIC, MBC, oxidative stress, peridontitis, green and purple complex

Summary

Introduction. Bacteria, by combining into specific groups according to the consumption of nutrients and the appropriate pH in the oral cavity, create specific complexes, thanks to which individual individuals can very effectively use nutrients and protect themselves well against the host's immune system. Gingival pockets are particularly exposed to the habitat existence of individual pathogenic strains of bacteria, especially if they are pathologically deepened. They then become niches in which organic debris and bacterial microorganisms settle. Socransky divided the microorganisms living in the oral biofilm into several groups, guided by the relationships between the species of microorganisms, and also assigning them appropriate colors. The complexes were distinguished in this way: red – include bacteria most pathogenic to periodontal tissues; orange – include bacterial species that are directly related to the microflora belonging to the red complex; yellow – include bacteria associated with shallow periodontal pockets; purple – formed by bacteria that are moderately pathogenic like microorganisms from the red and yellow complexes; blue – develops in people with persistent periapical changes; these include bacteria from the *Actinomycetes* family; green – has a significant impact on the development of caries.

Aim. The aim of the study was to test the antibacterial properties of pastes and gels from Indent – a leader in dental prophylaxis and distributor of companies such as Curasept, Tello, Frezyderm, which help accelerate the regeneration of gingival pockets and their healing, as well as prevent chronic inflammation in the oral cavity in the form of long infections or ulcerative aphthae. Protecting against caries and persistent periapical changes of periodontal tissues.

Material and methods. Reference strains of probiotic bacteria as a control *Bifidobacterium lactis* HNO 19, *Kluyveromyces marxianus fragilis* B0399, *Lactobactilius reuterii* Prodentis, *Lactobacillus salivarius* SGL03 and pathogenic strains of green and purple complexes. Green Complex bacteria in pockets above 6 mm: *Eikinella corrodens* (ATCC 23834),

Capnocytophaga gingivalis (ATCC 33624), *Agregatibacter* type a (ATCC 29522), *Agregatibacter* type b (ATCC 43718) and purple complex: *Vellionella parvula* (ATCC 17745), *Actinomyces odontolyticus* (ATCC 17929) were provided by (LGC Standards U.K.) and were used according to the recommendations of ISO 11133:2014. These strains were used to test the antibacterial activity with the analyzed compounds using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).
Results. The five compounds tested showed superselectivity and antimicrobial activity, in all analyzed bacterial strains of both green and purple complexes which had a profile similar to that obtained using currently used antibiotics, such as ciprofloxacin, bleomycin and cloxacillin, observed in MIC and MBC tests.
Conclusions. It should also be noted that the cost of the obtained compounds is low, which may be an attractive alternative to currently used antimicrobials. This will allow for a significant reduction in the costs of commonly used antibiotics and will contribute to the health-promoting properties of the entire organism.

Słowa kluczowe

MIC, MBC, stres oksydacyjny, zapalenie przyzębia, kompleks zielony i fioletowy

Streszczenie

Wstęp. Bakterie łącząc się w określone grupy względem zużywania składników pokarmowych oraz odpowiedniego pH w jamie ustnej, tworzą specyficzne kompleksy, dzięki którym poszczególne osobniki mogą bardzo skutecznie wykorzystywać składniki odżywcze i dobrze chronić się przed układem odpornościowym gospodarza. Szczególnie na bytowanie siedliskowe poszczególnych patogennych szczepów bakterii narażone są kieszenie dziąsłowe, zwłaszcza jeśli są patologicznie pogłębione. Stają się one wówczas niszami, w których osadzają się resztki organiczne i mikroorganizmy bakteryjne. Socransky podzielił mikroorganizmy żyjące w biofilmie jamy ustnej na kilka grup, kierując się relacjami między gatunkami mikroorganizmów, a także przypisując im odpowiednie kolory. Kompleksy wyróżniono w ten sposób: czerwone – obejmują bakterie najbardziej patogenne dla tkanek przyzębia; pomarańczowe – gatunki bakterii, które są bezpośrednio związane z mikroflorą należącą do kompleksu czerwonego; żółte – bakterie związane z płytkimi kieszonkami przyzębia; fioletowe – tworzą bakterie, które są umiarkowanie patogenne, jak mikroorganizmy z kompleksów czerwonego i żółtego; niebieskie – rozwija się u osób z uporczywymi zmianami okołowierzchołkowymi; obejmują one bakterie z rodziny promieniowców; zielone – ma istotny wpływ na rozwój próchnicy.
Cel pracy. Celem badania było sprawdzenie właściwości antybakteryjnych past i żeli firmy Indent – lidera w profilaktyce stomatologicznej i dystrybutora takich firm, jak: Curasept, Tello, Frezyderm, które pomagają przyspieszyć regenerację kieszonek dziąsłowych oraz ich gojenie, a także zapobiegać przewlekłym stanom zapalnym w jamie ustnej w postaci długich infekcji lub wrzodziejących aft, chroniąc przed próchnicą oraz uporczywymi zmianami okołowierzchołkowymi tkanek przyzębia.
Materiał i metody. Referencyjne szczepy bakterii probiotycznych jako kontrola *Bifidobacterium lactis* HNO 19, *Kluyveromyces marxianus fragilis* B0399, *Lactobactilius reuterii* Prodentis, *Lactobacillus salivarius* SGL03 oraz patogenne szczepy kompleksów zielonego i fioletowego. Zielony kompleks bakteryjny występuje w kieszonkach powyżej 6 mm a należą do niego nastepujace bakterie: *Eikinella corrodens* (ATCC 23834), *Capnocytophaga gingivalis* (ATCC 33624), *Agregatibacter* typ a (ATCC 29522), *Agregatibacter* typ b (ATCC 43718) oraz kompleksu fioletowego: *Vellionella parvula* (ATCC 17745), *Actinomyces odontolyticus* (ATCC 17929) zostały dostarczone przez (LGC Standards U.K.) i były używane zgodnie z zaleceniami normy ISO 11133:2014. Szczepów tych użyto do przetestowania aktywności przeciwbakteryjnej z analizowanymi związkami przy użyciu minimalnego stężenia hamującego (MIC) i minimalnego stężenia bakteriobójczego (MBC).
Wyniki. Przebadane pięć związków wykazało superselektywność i aktywność przeciwdrobnoustrojową we wszystkich analizowanych szczepach bakteryjnych kompleksów zielonego i fioletowego – miały profil podobny do uzyskanego przy użyciu obecnie stosowanych antybiotyków, takich jak cyprofloksacyna, bleomycyna i kloksacylina, obserwowanych w testach MIC i MBC.
Wnioski. Należy również zauważyć, że koszt uzyskanych związków jest niski, co może stanowić atrakcyjną alternatywę dla obecnie stosowanych środków przeciwdrobnoustrojowych. Pozwoli to znacznie obniżyć koszty powszechnie stosowanych antybiotyków oraz przyczyni się do właściwości prozdrowotnych całego organizmu.

Introduction

The microbial colonization depends on the availability of nutrients and the breaking of the natural limit of non-specific immunity. The first to inhabit the oral cavity are the so-called pioneer species transmitted by the macroorganism and obtained from the environment. They colonize specific ecological niches and, over time, multiply, creating ecological communities. The environment in which they live changes as a result of their metabolic activity, which facilitates the penetration of other bacterial species into them, and thus begins ecological succession, which leads to a large and significantly diversified environment of sessile microflora, or to pathological changes (1-17).

The colonization of the tooth surface by microorganisms is possible due to adhesion or adherence processes. Adhesins enable the connection of the lipophilic surface of bacterial cells with the hydrophobic surface of epithelial cells. Adhesins are proteins that bind to carbohydrates using structures called fimbriae (fibrils of various lengths found on the cell surface). Fimbriae have been detected on streptococci: *S. anginosus*, *S. salivarius*, *S. oralis*, *S. mutans*, on *Actinomycetes – A. viscosus*, *A. naeslundi* and in Gram-negative rods – *Prevotella*, *P. intermedia*, *P. oralis*, *P. bucceae*, *P. melaniogenica* and the species *Porphyromonas gingivalis* (18-20).

Other adhesive substances are substances that stick together or transport substrates, e.g. polypeptides that bind salivary glycoproteins to bacterial cells or calcium ions. Extracellular polypeptides that react with streptococcal proteins behave similarly. The cell walls of streptococci contain lipoteichoic acids that can bind to the acquired salivary sheath (a thin layer that forms from salivary glycoproteins on teeth just a few minutes after brushing and adheres to them). An important role in colonization processes is played by cytoplasmic membrane polyproteins that act as a "transporter" of components, e.g. sugar or peptides associated with the acquired salivary sheath or the surfaces of other bacterial cells. So-called ligands take part in the adhesion processes – negatively charged salivary glycoproteins that can occur on epithelial cells of the mucous membrane, hard tissues of the tooth above and below the gums and on prosthetic restorations. These compounds are positively charged (Ca^{+2}), so they bind to negatively charged bacterial adhesins of streptococci – *S. mutans*, *S. oralis*, Actinomyces rods, Gram-negative bacteria, i.e. *P. intermedia*, *F. nucelatum*, *P. gingivitis*, *E. corrdens* (1-13, 18-20).

Another important adhesion mechanism is bacterial aggregation and colonization process, which involves the attachment of microorganisms to each other and their adherence to various surfaces. The main substrates in the oral cavity are: the acquired membrane on the hard surfaces of enamel and root cement (bones) and on the oral mucosa. The membrane covering the surfaces is about 1 micron thick and is characteristic of each surface. The membranes on the surfaces of hard tissues are not identical; on the epithelium they are referred to as the mucosal membrane (5, 13, 20-33).

In the oral cavity, bacteria group into specific groups, also known as complexes, thanks to which they use nutrients better and defend themselves more effectively against the defense mechanisms of the macroorganism (32, 33). The concept of "bacterial complex" was introduced by Socransky (34). The division criterion is the relationship between bacteria. Socransky divided pathogens in the biofilm into 6 groups, to which he assigned an appropriate color – blue, yellow, green, purple, orange and red. The last three complexes (purple, orange and red) dominate in the subgingival space (34-48). In our studies, we focused on bacteria of the green and purple complexes (fig. 1).

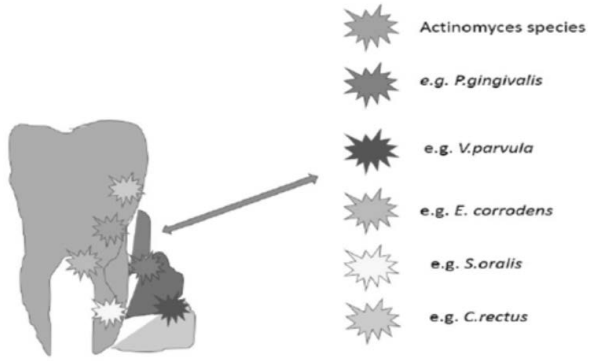

Fig. 1. Bacterial complexes in the oral cavity according to Socransky (34)

The green group consists of *Eikenella corrodens* is a Gram-negative bacteria found in the upper respiratory tract and the digestive tract of humans. It constitutes the physiological bacterial flora of the periodontium. It was identified in 1958 by M. Eiken and named *Bacteroides corrodens* (48). It is a pleomorphic cell (often takes the form of a rod), Gram-negative. It is a relative anaerobe, it grows better in an atmosphere enriched with 5-10% CO_2. It has high growth requirements – it grows on blood agar and chocolate agar (48). It grows in the form of light yellow colonies, often with a depression in the center. It does not produce catalase, it produces cytochrome oxidase (49). It belongs to the HACEK group (40) (slow-growing Gram-negative bacteria constituting the physiological flora), which can cause endocarditis in children. This group also includes: *Haemophilus* sp., *Actinobacillus actinomycetemcomitan*, *Cardiobacterium hominis*, *Kingella kingae* (50). *Eikenella corrodens* is resistant to: macrolides, aminoglycosides, lincosamides, metronidazole and intermediately susceptible to quinolones (51).

The genus *Capnocytophaga* is a group of spindle-shaped, Gram-negative, rod-shaped organisms with gliding motility. These organisms are part of the normal oral flora and were first described as *Bacteroides ochraceus* and CDC group DF-1. In patients without evidence of immune defects, cellulitis or wound infection occurs. Infections caused by *Capnocytophaga* can be treated with antibiotics, but

complications of the disease can occur rapidly and can be potentially fatal. *Aggregatibacter actinomycetemcomitans* is a bacterium commonly associated with aggressive periodontitis, most severe in the central incisors and first molars. As in previous classifications, the occurrence of this organism correlated with juvenile periodontitis, and to a lesser extent with rapidly progressive periodontitis. It is a Gram-negative, nonmotile, facultatively anaerobic rod, as the name suggests, associated with actinomycetes, belonging to the family *Pasteurellaceae*. Based on the structure of membrane polysaccharides, six basic serotypes of this pathogen have been distinguished, of which three occur most frequently in the oral cavity, marked with the letters a, b and c (51). According to a study by Perry et al. (51) serotypes a and b are detected in almost equal proportions in healthy individuals and those with chronic periodontitis (then referred to as adult periodontitis), serotype b occurs almost twice as often in aggressive periodontitis (then referred to as juvenile periodontitis), serotype c appears less frequently, in equal proportions, and is primarily responsible for infections outside the oral cavity. The most common such infections are brain abscesses, osteitis and endocarditis (51).

Veillonella parvula is a strictly anaerobic, Gram-negative bacterium, resembling a granuloma (52). It is a natural part of the oral flora, but as a result of changes in pH in the oral cavity it can participate in periodontitis and tooth decay, as well as systemic infections, which include meningitis, osteomyelitis (53). Along with *Gardanella* it can participate in bacterial vaginosis in women. It can also be associated with arterial hypertension together with *Campylobacter rectus* and *Prevotella* melaninogenica (54). *Veillonella parvula* feeds on lactates provided by *Streptococcus* species, present in the oral cavity (55). *Streptococcus* mutans and *Veillonella parvula* can form multi-species biofilms, which lead to reduced susceptibility to antibacterial treatment in the oral cavity, which can lead to tooth decay and periodontitis (56). Bacteria of the genus *Veillonella* are sensitive to metronidazole and penicillin (57, 58). Active antibiotics against *Veillonella parvula* are cephalosporin, clindamycin and chloramphenicol (57).

Actinomyces odontolyticus was first described in the 20th century (59). In humans, these bacteria occur mainly on the mucous membranes of the oral cavity, gastrointestinal tract and female reproductive organs. After tissue disruption, bacteremia, sepsis and endocarditis often develop (60). *Actinomyces odontolyticus* can induce individual cases of infection leading to meningeal or brain abscesses (61-64).

Aim

The aim of the study was to test the antibacterial properties of pastes and gels from Indent – a leader in dental prophylaxis and distributor of companies such as Curasept, Tello, Frezyderm, which help accelerate the regeneration of gingival pockets and their healing, as well as prevent chronic inflammation in the oral cavity in the form of long infections or ulcerative aphthae. Protecting against caries and persistent periapical changes of periodontal tissues.

Material and methods

Microorganisms and media

Reference strains of probiotic bacteria as a control *Bifidobacterium lactis* HNO 19, *Kluyveromyces marxianus fragilis* B0399, *Lactobactilius reuterii* Prodentis, *Lactobacillus salivarius* SGL03 and pathogenic strains of green and purple complexes. Green Complex bacteria in pockets above 6 mm: *Eikinella corrodens* (ATCC 23834), *Capnocytophaga gingivalis* (ATCC 33624), *Agregatibacter* type a (ATCC 29522), *Agregagibacter* type b (ATCC 43718) and purple complex: *Vellionella parvula* (ATCC 17745), *Actinomyces odontolyticus* (ATCC 17929) were provided by (LGC Standards U.K.) and were used according to the recommendations of ISO 11133:2014. These strains were used to test the antibacterial activity with the analyzed compounds using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as described in (21, 47).

MIC and MBC tests

The MIC was estimated by a microtiter plate method using sterile 48 or 96-well plates (47). First, precursor and TIL solutions were prepared sterile deionized water at 20 mg mL^{-1}. Fifty microliters of the solutions was placed in the first row of the plate. Next, 25 µL of sterile TSB medium was added to the other wells, and serial dilutions were performed. Then, 200 µL of inoculated TSB medium containing resazurin (0.02 mgmL^{-1}) as an indicator was added to all the wells. TSB medium was inoculated with 106 colony-forming units (CFU) mL^{-1} (approximately 0.5 McFarland units) of the bacterial strains. The plates were incubated at 30°C for 24 h. Color changes from blue to pink or yellowish with turbidity were taken as positive, and the lowest concentration at which there was no visible color change was the MIC. The MBC was estimated based on the measurement of the dehydrogenases activity in the cultures after a 24-h incubation without the ILs. Four millilitres of a dense culture (approximately 109 CFU mL^{-1}) that was incubated for 24 h in TSB medium at 25°C was added to identical test tubes. Next, the tested compounds were added to the test tubes until the mixture reached final concentrations of 10-250 mg mL^{-1}. Then, the cultures containing the TILs were incubated for 1 h at 30°C. Next, 0.1 g of $CaCO_3$ and 0.1 mL of a 3% triphenyltetrazolium chloride (TTC) solution were added to the test tubes. Then, the test tubes were sealed with parafilm and incubated for 1 h 30°C in darkness.

MTT-assay

The cytotoxic effects of the tested peptidomimetics 1-16 on BALB/c3T3 mouse fibroblast cells, after 24 h of incubation at a concentration of 1 mmol/mL, were determined by MTT assay. The MTT test is based on the ability

of the enzyme mitochondrial dehydrogenase to convert an orange, water-soluble tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into insoluble forma-zan, which is a dark blue product of the above reaction. After dissolving formazan crystals in DMSO or isopropanol, a coloured solution is formed, the intensity of which is measured spectrophotometrically in the wavelength range of 492-570 nm. The amount of coloured reduced MTT is proportional to the oxidative activity of the cell's mitochondria, and under strictly defined experimental conditions to the number of metabolically active (living) cells in the population. The MTT test can also be used to determine cell viability in populations of cells that no longer divide but are metaboli-cally active. The MTT test is currently the most commonly used to assess cytotoxic activity and is recommended as a reference by international standards-setting organisations (65).

Statistical analysis

All experimental data from at least three different trials (n = 3) are given as means ± standard error of the mean (SEM, manufacturer, Saint Louis, MO, USA). To compare pairs of means, the Tukey post hoc test was used, indicating statistical significance with * $p < 0.05$,** $p < 0.1$, and *** $p < 0.01$ (10).

Analysed compounds – characteristics

All counds were used with manufacturer recommendation

1. CURASEPT DAYCARE HERBAL INVASION − protective toothpaste with CPC-HAP 75 ml

Curasept Daycare Protection Booster toothpaste contains essential oils with innovative cetylpyridinium chloride and hydroxyapatite complex (CPC-HAP), whose synergistic action prevents the multiplication of microorganisms in the oral cavity for up to 4 hours after use. Contains a herbal flavor. It is SLS-free. Contains 900 ppm of fluoride. Fights caries and gum disease, halitosis, disinfects and remineralizes:

Active ingredients of Curasept Daycare Herbal Invasion toothpaste:

1) Cetylpyridinium chloride − Has an antiseptic effect. 2) CPC-HA Complex (Cetylpyridinium Chloride + Hydroxyapatite) − Hydroxyapatite creates a protective layer that remineralizes while gradually releasing cetylpyridine, providing extended protection for up to 4 hours. 3) Essential oils (menthol, eucalyptol, thymol, methyl salicylate) − Prevent bacterial aggregation and slow down the growth of biofilm in the oral cavity. Penetrate the dental plaque and have a long-lasting effect at the supragingival and subgingival levels. They also help prevent gingivitis, help with daily mechanical plaque control and fight bad breath. Basic composition: Aqua, Sorbitol, Hydrated Silica, Xylitol, Propylene Glycol, Peg-32, Cellulose Gum, Cocamidopropyl Betaine, Hydroxyapatite, Calcium Glycerophosphate, Sodium Fluoride, Cetylopiyridinium Chloride, Calcium Phantothenate, 1,2-hexanediol, Potassium Nitrate, Cetraria Islandica Extract, Thymol, Elettaria Card amomum Seed Oil, Eucalyptol, Menthol, Methyl Salicilate, Stevia Rebaudiana Leaf/stem Extract, Sodium Saccharin, Aroma, Propanediol, Xanthan Gum, Phenoxyethanol, Ethylhexylglycerin, P-anisic Acid, Sodium Benzoate, Chlorphenesin, Tetrasodium Glutamate Diacetate, C.l. 19140, C.l. 42090.

2. CURASEPT ADS 100 − 1% chlorhexidine gum gel 30 ml

The gum gel contains 1% chlorhexidine digluconate, which is one of the best ingredients on the market in effectively combating daily plaque. It also contains Purified water, Propylene Glycol, Hydroxy Ethyl Cellulose, PVP/VA copolymer, PEG-40 hydrogenated castor oil, Chlorhexidine digluconate, Sodium acetate, Aroma, Acetic acid, Sodium metabisulfite, Ascorbic acid.

Using the gel protects the gums and interdental spaces, and also prevents infection of the oral cavity by pathogens, which may be bacteria, fungi or viruses. In addition, the gel has a healing effect on ulcers in the oral cavity, treats periodontal disease, and also combats inflammation caused by irritation of the dental prosthesis. The ADS 100 gel in a 30 ml package is distinguished by its highly concentrated 1% chlorhexidine which acts radically against bacteria and dental plaque. It acts locally in the dental pocket for up to 12 hours after application. And also in the local treatment of gums during dental treatment, for owners of dentures, orthodontic appliances. It is also intended for patients with periodontal disease, having dental implants and also having a tendency to excessive accumulation of dental plaque. It does not change the taste of consumed food and drinks. The CURASEPT A.D.S. function slows down and inhibits the formation of bacterial plaque and prevents irritation of the gums and oral cavity.

3. CURASEPT PREVENT – toothpaste 75 ml

Curasep Prevent is a toothpaste designed for prophylaxis and maintaining the health of gums and peri-implant tissues. Moreover, it helps maintain the effect of dental therapy in patients with chronic problems or during dental procedures. The synergistic selection of ingredients means that the paste helps maintain the ideal oral microflora. Additionally, Curasept Prevent paste hinders the multiplication of the most aggressive microorganisms, restores the balance of bacterial flora and improves the health of gum and periodontal tissues, while counteracting halitosis. CURASEPT PREVENT toothpaste is intended especially for: diabetics, smokers, people suffering from metabolic diseases, with dry mouth, with weakened immunity, undergoing chemotherapy and radiotherapy, during orthodontic and implant treatment, patients (with complex prosthetic rehabilitation) treated for periodontal problems in the maintenance phase. Contains Sorbitol, Aqua, Hydrated Silica, Xylitol, PEG-32, Sodium Lauroyl Sarcosinate, Sodium Cocoyl Glutamate, VP-VA Copolymer, Cellulose Gum, Phenoxyethanol, Sodium

Saccharin, Sodium Citrate, Sodium Benzoate, Hexetidine, Ozonized Olive Oil, Melaleuca Alternifolia Leaf Oil, Stevia Rebaudiana Leaf/Steam Extract, Colostrum, Aroma, C.I. 19140, C.i. 42090.

a) Ozonated Oil – Gradually releases ozone and creates a favorable micro-environment for maintaining the balance of oral microflora, as well as improving the health of tissues and mucous membrane.

2) Colostrum – Supports regeneration and strengthens gums and oral mucosa, thanks to the content of enzymes and proteins with a defensive effect that interact with lactoferrin and lysozyme.

3) PVP/VA – Thanks to its ability to adhere to teeth and mucous membrane, it creates a protective layer, hindering the adhesion and proliferation of bacterial plaque and delays the formation of biofilm.

4) Tea Tree Oil – Contains a natural set of substances with antioxidant action. Effectively prevents the formation of biofilm in the oral cavity.

5) Stevia – Natural sweetener with anti-caries effect. Widely used to inhibit the formation of biofilm in the oral cavity.

4. CURASEPT PREVENT – oral gel 30 ml

Curasep Prevent is an oral gel indicated for the protection, prevention and maintenance of implants and control of risky situations related to periodontal disease. Thanks to its ability to adhere to teeth and mucous membrane, it creates a protective coating that inhibits the adhesion and proliferation of bacterial plaque and the formation of biofilm. Additionally, it improves the health of gum tissue, even in the case of comorbidities and balances the oral microflora.

Active ingredients of the gel:

a) Ozonated Oil – Gradually releases ozone and creates a favorable micro-environment for maintaining the balance of oral microflora, as well as improves the health of tissues and mucous membrane.

b) Colostrum – Supports regeneration and strengthens gums and oral mucosa, thanks to the content of enzymes and proteins with a defensive effect that interact with lactoferrin and lysozyme.

c) PVP/VA – Thanks to its ability to adhere to teeth and mucous membrane, it creates a protective layer, hindering the adhesion and proliferation of bacterial plaque and delays the formation of biofilm.

d) Tea tree oil – contains a natural set of substances with antioxidant action. Effectively prevents the formation of biofilm in the oral cavity.

e) Stevia – a natural sweetener with anti-caries action. Widely used to inhibit the formation of biofilm in the oral cavity.

Restores the balance of bacterial flora and improves the health of gum tissues, also in the case of comorbidities. Ideal for patients with implants, smokers, diabetics, with dry mouth and patients with weakened immune resistance. Prevents halitosis.

5. CURASEPT ADS 350 – office set of gels for gums with chlorhexidine 0.5% – 5 ml

The gel for gums contains chlorhexidine digluconate in a concentration of 0.5%, which is one of the best ingredients on the market in the effective fight against daily plaque. Using the gel protects the gums and interdental spaces, and also prevents infection of the oral cavity by pathogens, which may be bacteria, fungi or viruses. Additionally, the gel has a healing effect on ulcers in the oral cavity, treats gingivitis, and also combats inflammation caused by irritation of the dental prosthesis. Curasept ADS 350 gel is intended for patients after complicated extraction, after maxillofacial surgery, with post-bite trauma and mucosal damage with ulcers and aphthous stomatitis, with mucosal infections.

Contains A.D.S.® System – ANTI DISCOLORATION SYSTEM

Composition of the gel: Aqua, Propylene Glycol, Xylitol, Hydroxyethyl Cellulose, Chlorhexidine Digluconate, Ascorbic Amide. Peg-40 Hydrogenated Castor Oil, Sodium Metabisulphite, Aroma, Methylparaben

Results

The effects of the 5 compounds investigated (illustrated in fig. 3-5) were evaluated within bacterial cells using a previously established methodology (21, 47). Following the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), the MIC values ranged between 0.5-1.5 µM, while the MBC values fell within the range of 1.5-2.5 (+/- 0.5) µM for analysed for four bacterials strains used as control and, Gram-negative strains and Staphylococcus, as depicted in figure 4. MIC serves as a reference for assessing the susceptibility or resistance of bacterial strains to the antibiotic applied in vitro. On the contrary, MBC represents the minimum concentration of an antibacterial agent required to eradicate bacteria, distinguishing it as bactericidal rather than bacteriostatic (34, 47). Basically, all compounds tested against Gram-positive and Gram-negative bacterial strains show good activity against them. And all MIC values are below 1.5 $\mu g/mL^{-2}$ (fig. 2). Interestingly, the change in the different compounds had a vital impact on activity. However, the fluorine compounds and other active ingredients was playing a role in the activity against some bacteria, which we confirmed with the activity of compound no. 1 and 5. This means that the compounds had a good potency compared to the analysed antibiotics (fig. 6). Interestingly, all five compounds showing broad spectrum activity against both Gram-negative as well as Gram-positive bacteria. The steric hindrance created by the bulky fluorine atom and other active ingredients might prevent proper binding and interaction with the active site residues, thereby reducing the compound's overall efficacy. The analyzed probiotic strains were resistant to the action of pastes and gels, while the pathogenic strains were destroyed by strong cytotoxic properties damaging both Gram-negative and Gram-positive

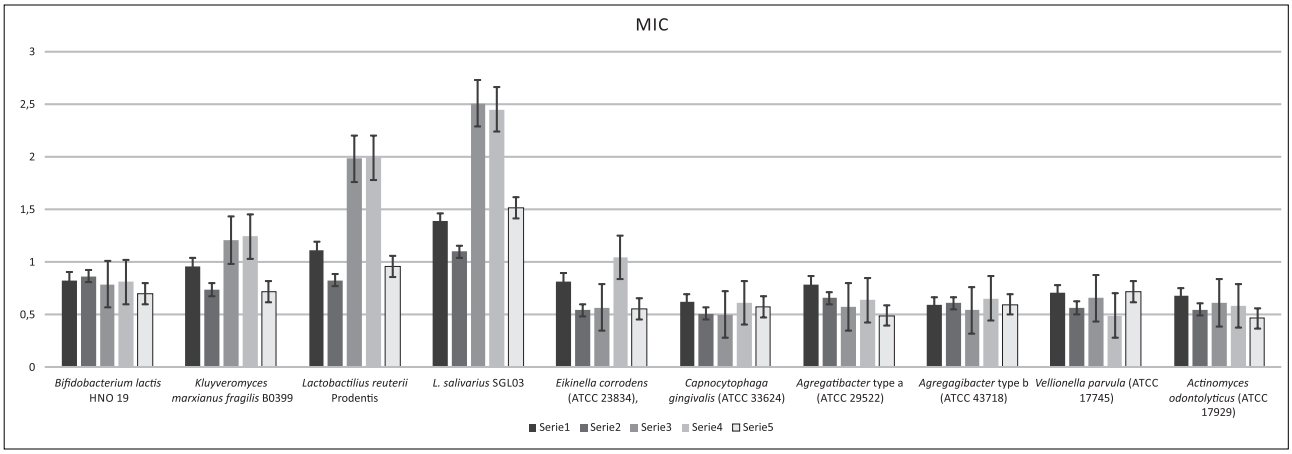

Fig. 2. Minimum inhibitory concentration (MIC) of the coumarin derivatives in model bacterial strains. The x-axis compounds 1-5 used sequentially. The y-axis shows the MIC value in μg/mL^{-1}

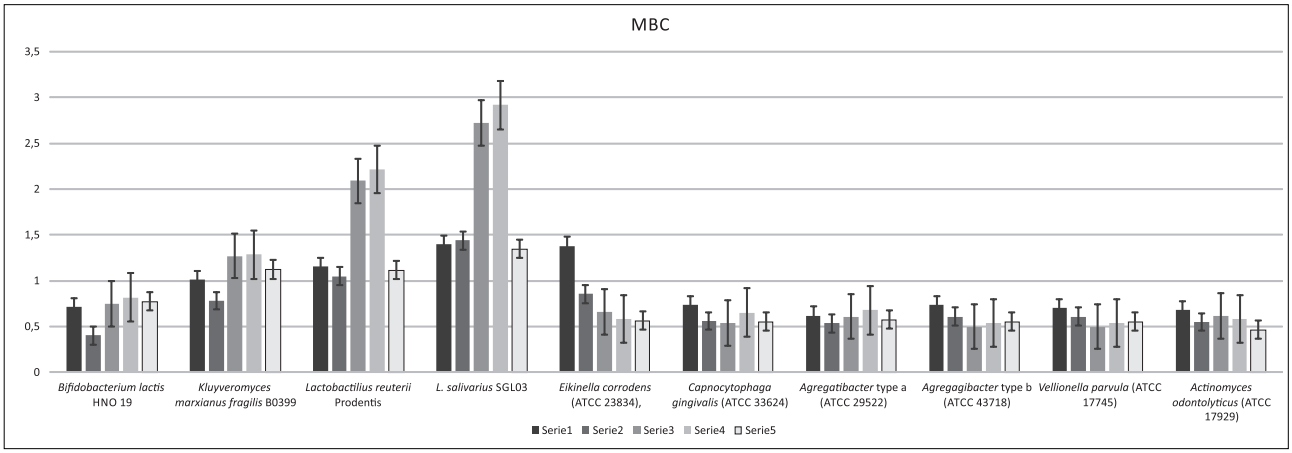

Fig. 3. Minimum bactericidal concentration (MBC) of coumarin derivatives in model bacterial strains. The x-axis features compounds 1-5 used sequentially. The y-axis shows the MBC value in μg/mL^{-1}

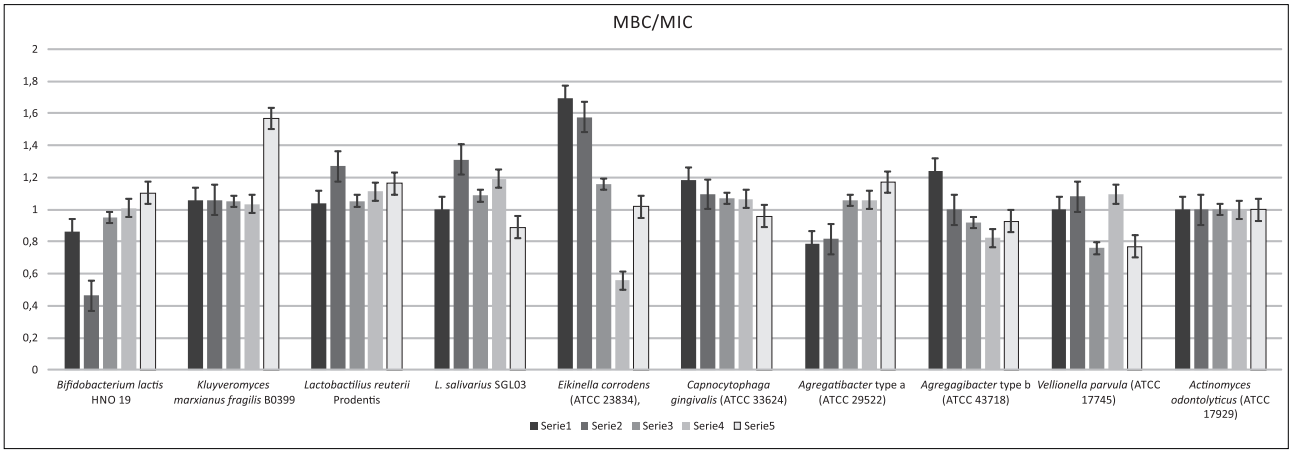

Fig. 4. The ratio of MBC/MIC of the investigated agents 1-16 in selected bacterial strains. The x-axis compounds 1-5. The y-axis shows the MBC/MIC value in μM shows the MIC value in μM

pathogens. This indicates their selective action on red and yellow complex bacteria.

Antimicrobial agents are often classified as either bacteriostatic or bactericidal. A medicine is deemed bactericidal if the MBC-MIC ratio is low (less than 1.5), and it is feasible to achieve drug concentrations that eradicate 99.9% of the exposed organisms. Should the proportion of antibiotic be classified as bacteriostatic; nevertheless, the MBC to MIC ratio is high and it may not be safe to take doses of the medication to kill 99.9% of bacteria. The exact distinction between the bacteriostatic and bactericidal properties of many substances depends on the concentration of the

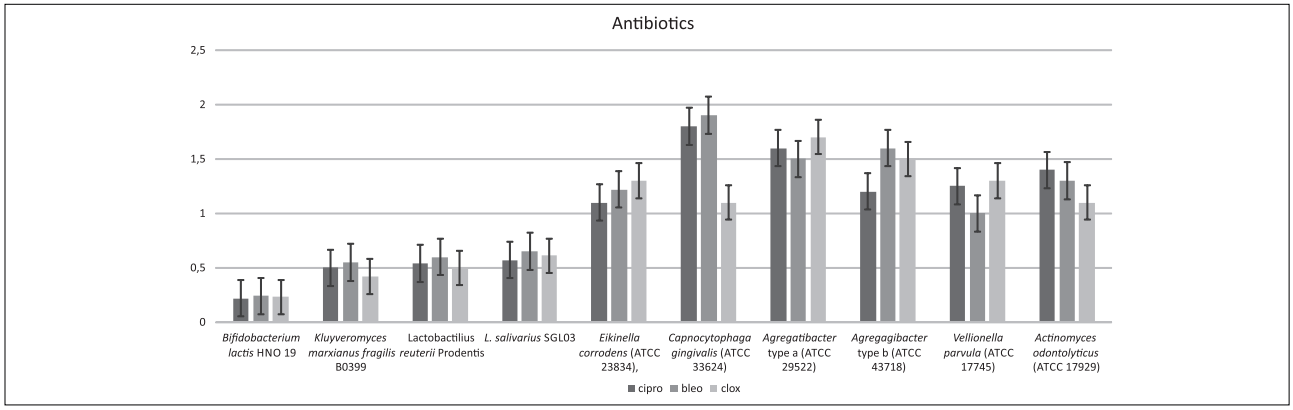

Fig. 5. Examples of MIC in selected bacterial strains for studied antibiotics ciprofloxacin (cipro), bleomycin (bleo), and cloxacillin (clox). The x-axis features antibiotics used sequentially. The y-axis shows the MIC value in μM.

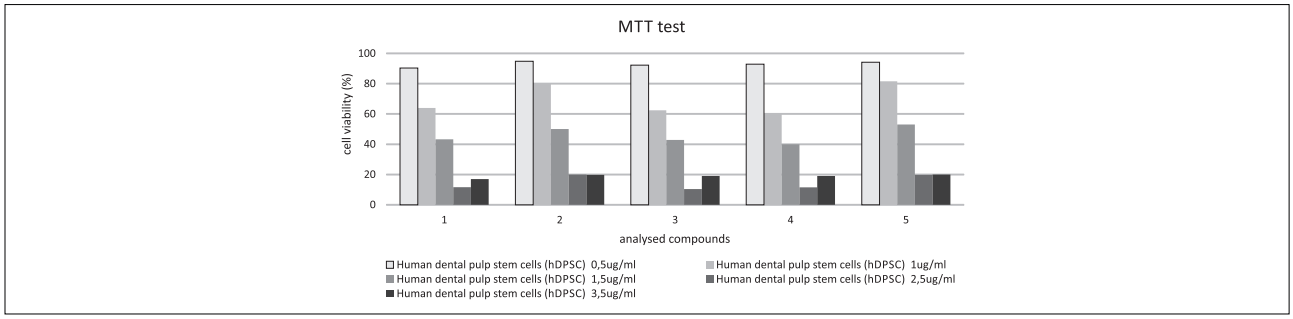

Fig. 6. Measurement of cell viability (%) (y-axis) on human dental pulp stem cells (hDPSC) when exposed to analysed compounds 1 – during 24 hours incubation. The x-axis features compounds 1-5

Tab. 1. Statistical analysis of all analyzed compounds by MIC, MBC, and MBC/MIC; < 0.05*, < 0.01**, < 0.001***

No. of Samples	1, 2	3	4-5	Type of test
Bifidobacterium lactis HNO 19	*	**	**	MIC
Kluyveromyces marxianus fragilis B0399	*	**	**	MIC
Lactobactilius reuterii Prodentis	*	**	**	MIC
Lactobacillus salivarius SGL03	*	**	**	MIC
Eikinella corrodens (ATCC 23834)	*	*	*	MBC
Capnocytophaga gingivalis (ATCC 33624)	*	*	*	MBC
Agregatibacter type a (ATCC 29522)	*	*	*	MBC
Agregagibacter type b (ATCC 43718)	*	*	*	MBC
Vellionella parvula (ATCC 17745)	*	*	*	MIC
Actinomyces odontolyticus (ATCC 17929)	*	–		MIC

pathogen and the drug that is reached in the target tissue. In the case of all tested compounds 1-5, we are dealing with bactericidal agents (fig. 4).

Furthermore, as can be seen in figures 3 and 5, investigated compounds typically exhibited greater antibacterial activity than commonly used antibiotics. This is especially crucial because tested microorganism resistance to known antibiotics has evidently increased, such as cloxacillin (clox) or ciprofloxacin (cipro) (fig. 6). The rate at which microorganisms will become resistant to bleomycin (bleo) is unknown, but this will cause a major disruption in the antibiotic arsenal that is now used to treat hospital infections. Interestingly, our compounds exhibit activity comparable to that of bleomycin (bleo).

Model of analysed strains with tested compounds were statis-tical significance at $p < 0.05$ (tab. 1).

The Human dental pulp stem cells (hDPSC) (as good model for cell observed and obtained in oral cavity) were treated with tested compounds with concentrations ranging from 1 to 3.5 μg/mL and were incubated for 24 hours. All tested compounds were not cytotoxic in analysed cells at the lowest concentrations tested of 1 μg/mL, the viability

percentages remains above 99.50%. However, gradual reduction in viability was caused by tested 5 agents at 2 µg/mL ranging the cell viability percentages from 87.00% for compound 5 and 66.50% respectively. The obtained results were used to calculate the half-maximal inhibitory concentration (IC50) after 24 hours of incubation with selected most active antimicrobial used oral cavity compounds 1-5. The IC50 value for hDPSC after 24 h of incubation ranging from 3.22 µg/mL for compound 1 up to 3.26 µg/mL for all compounds respectively.

Discussion

Cytotoxic studies of tested compounds

The effects of the 5 compounds investigated (illustrated in fig. 3-5) were evaluated within bacterial cells using a previously established methodology (21, 47). Following the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), the MIC values ranged between 0.25-4.5 µM, while the MBC values fell within the range of 1-8 (+/- 0.5) µM for bacterial strains of green and purple complexes; *Eikinella corrodens* (ATCC 23834), *Capnocytophaga gingivalis* (ATCC 33624), *Agregatibacter* type a (ATCC 29522), Agregagibacter type b (ATCC 43718) and purple complex: *Vellionella parvula* (ATCC 17745), *Actinomyces odontolyticus* (ATCC 17929) *Bifidobacterium lactis* HNO 19, *Kluyveromyces marxianus fragilis* B0399, *Lactobactilius reuterii* Prodentis, *Lactobacillus salivarius* SGL03.

MIC serves as a reference for assessing the susceptibility or resistance of bacterial strains to the antibiotic applied *in vitro*. On the contrary, MBC represents the minimum concentration of an antibacterial agent required to eradicate bacteria, distinguishing it as bactericidal rather than bacteriostatic (47). Basically, all our compounds tested against Gram-positive and Gram-negative bacterial strains show good activity against them. And all MIC values are below 5 $\mu g/mL^{-1}$ (fig. 3). Interestingly, the change in the different toothpaste and gels had a vital impact on activity. The oral hygiene products containing active ingredients in their composition was playing a role in the activity against some bacteria, which we confirmed with the activity of compound no. 1 and 5. This means that the active comonents had a good potency compared to antibiotics. Interestingly, all compounds showing broad spectrum activity against both Gram negative as well as gram positive bacteria but the highest the compound no 3 and 4. Compounds 1 to 5 have greater efficacy against: *Eikinella corrodens* (ATCC 23834), *Capnocytophaga gingivalis* (ATCC 33624), *Agregatibacter* type a (ATCC 29522), *Agregagibacter* type b (ATCC 43718) and purple complex: *Vellionella parvula* (ATCC 17745), *Actinomyces odontolyticus* (ATCC 17929) strains. Compounds No. 3 and 4 demonstrated a broad range and good action against all bacteria, as revealed confi-dentially with respect to the fluorine group. Furthermore, compounds with a para-fluoro group in had more activity overall. The obtained results were a good and very simple and cheap training test which allowed us to estimate to what extent the types of specific diets have an influence on the formation of specific bacterial biofilms on the induction of inflammatory conditions of periodontal disease with specific bacterial biofilms. The methods described in the manuscript significantly shorten the time of detection of disease entities induced by persistent bacterial biofilms belonging to different classes. They also allow the assessment of the actual inflammation of the periodontal tissues. By specifying the type of identifier, the doctor can directly estimate what type of bacteria he is dealing with and what treatment should be administered. The obtained results suggest that the substances contained in the analysed components may also interact with red complexes of bacteria showing cariogenic activity present in the human oral cavity (11, 12). Currently, antibiotic resistance among pathogenic bacteria is becoming more and more common, leading to super-resistance. The lack of colonization capabilities by such pathogens is the result of the lack of access to adhesins occupied by the microflora of the inhabited niche. Moreover, the sessile microflora can produce certain antagonistic compounds that interfere with the colonization of the niche by exogenous bacterial strains, e.g. hydrogen peroxide produced by *S. mitis*, salivaricin produced by *S. salivarius streptococci* (which inhibits the development of pyogenic streptococci *S. pyogenes*) or enocin (bacteriocin) produced by *S. mutans*, *C. martuchotii* and *A. actinomycetemcomitans* (which inhibits the growth of related organisms) of yellow and red complexes (36).

Conclusions

The observed results are especially important because of increasing resistance of bacteria to various drugs and antibiotics. All selected compounds showed super-selectivity in all analyzed bacterial strains and exhibited the highest cytotoxic activity, comparable or better than the commonly used antibiotics: ciprofloxacin, bleomycin, and cloxacillin. The bacterial flora of the oral cavity contributes to the development or aggravation of oxidative stress. As mentioned above, bacteria inhabiting the crowns of teeth breathe oxygen (Eh +200 mV) and produce carbon dioxide, which results in a decrease in the oxidative reduction potential to about +76 mV. As the bacterial plaque grows, this potential can decrease to as much as -140 mV. In the gingival crevices and pockets – in the subgingival dental plaque, the oxidative reduction potential fluctuates in the range of Eh +72 mV to -300 mV, which leads to the creation of an anaerobic environment. People with high levels of oxidative stress suffer from periodontal diseases twice as often and vice versa – periodontal diseases closely correlate with an increase in the level of oxidative stress parameters (22-26). The analyzed probiotic strains were resistant to the action of pastes and gels, while the pathogenic strains were destroyed by strong cytotoxic properties damaging both

Gram-negative and Gram-positive pathogens. This indicates their selective action on red and yellow complex bacteria. The analyzed ingredients of pastes and gels act selectively on bacterial pathogens without affecting human dental pulp stem cells (hDPSC), thanks to which the human pulp is protected by the compounds used, which is an invaluable value in maintaining healthy enamel and teeth during daily use in oral hygiene.

Conflict of interest
Konflikt interesów

None
Brak konfliktu interesów

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