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Analysis of the antimicrobial activity of ingredients contained in Curasept pastes, gels and rinses

Analiza aktywności przeciwdrobnoustrojowej składników zawartych w pastach, żelach i płukankach Curasept

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Keywords

MIC, MBC, oxidative stress, peridontitis

Summary

Introduction. Plaque build-up is a common cause of the first stage of gum disease – gingivitis. Plaque bacteria constantly accumulate on and around teeth. If plaque is not removed by regular tooth brushing, it can irritate the gums, causing them to become red and inflamed. The inflammation associated with gum disease is not painful, but if left untreated, it can progress and develop into periodontitis, a more serious, irreversible stage of gum disease that can ultimately lead to tooth loss. In addition, inflammation may cause canker sores, i.e. red, white or gray sores, which may be painful and may appear anywhere in the mouth, including on the gums, causing pain and inflammation. Canker sores can occur singly or appear simultaneously in different places in the mouth. Although unpleasant, they are usually harmless and disappear after a few days.
Aim. The aim of the study was to check the antibacterial properties of mouthwashes recommended by Indent – a leader in dental prophylaxis and distributor of companies such as Curasept, Tello, Frezyderm Curaprox, AloeFresh, which help accelerate healing and prevent persistent infections or recurrent canker sores.
Material and methods. The reference bacterial strains of *E. coli* (K12 ATCC 25404, R2 ATCC 39544, R3 ATCC 11775, R4 ATCC 39543), *Staphylococcus aureus* strain (ATCC 23235), as well as on *Acinetobacter baumannii* (ATCC 17978), *Pseudomonas aeruginosa* (ATCC 15442), *Enterobacter cloacae* (ATCC 49141) were provided from (LGC Standards U.K.) and were used according to the recommendation of ISO 11133:2014. These strains were used to test antibacterial activity with analyzed compounds by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).
Results. The tested compounds, showed an antimicrobial activity profile similar to that obtained with currently used antibiotics such as ciprofloxacin, bleomycin, and cloxacillin observed in MIC and MBC tests. It should also be noted that the cost of the compounds obtained is low, what may be an attractive alternative to the currently used antimicrobial agents.
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.

Słowa kluczowe

MIC, MBC, stres oksydacyjny, próchnica zębów

Streszczenie

Wstęp. Nagromadzenie płytki nazębnej jest częstą przyczyną pierwszego etapu choroby dziąseł – zapalenia dziąseł. Bakterie płytki nazębnej stale gromadzą się na zębach i wokół nich. Jeśli płytka nazębna nie zostanie usunięta podczas regularnego szczotkowania zębów, może podrażnić dziąsła, powodując ich zaczerwienienie i stan zapalny. Zapalenie związane z chorobą dziąseł nie jest bolesne, ale jeśli nie zostanie leczone, może postępować i przekształcić się w zapalenie przyzębia, poważniejszy, nieodwracalny etap choroby dziąseł, który może ostatecznie doprowadzić do utraty zębów. Ponadto stan zapalny może powodować afty, tj. czerwone, białe lub szare owrzodzenia, które mogą być bolesne i pojawić się w dowolnym miejscu w jamie ustnej, w tym na dziąsłach, powodując ból i stan zapalny. Afty mogą występować pojedynczo lub pojawiać się jednocześnie w różnych miejscach w jamie ustnej. Chociaż nieprzyjemne, są zwykle nieszkodliwe i znikają po kilku dniach.

Cel pracy. Celem badania było sprawdzenie właściwości antybakteryjnych płynów do płukania jamy ustnej rekomendowanych przez Indent – lidera w profilaktyce stomatologicznej i dystrybutora takich firm, jak: Curasept, Tello, Frezyderm Curaprox, AloeFresh, które pomagają przyspieszyć gojenie i zapobiegają uporczywym infekcjom lub nawracającym aftom.

Materiał i metody. Do badań zostały zastosowane referencyjne szczepy bakterii *E. coli* (K12 ATCC 25404, R2 ATCC 39544, R3 ATCC 11775, R4 ATCC 39543), szczep *Staphylococcus aureus* (ATCC 23235), a także *Acinetobacter baumannii* (ATCC 17978), *Pseudomonas aeruginosa* (ATCC 15442), *Enterobacter cloacae* (ATCC 49141) zostały dostarczone przez (LGC Standards U.K.) i były używane zgodnie z zaleceniami normy ISO 11133:2014. Szczepy te użyto do przetestowania aktywności przeciwbakteryjnej analizowanych związków przy minimalnym stężeniu hamującym (MIC) i minimalnym stężeniu bakteriobójczym (MBC).

Wyniki. Badane związki wykazały profil aktywności przeciwdrobnoustrojowej podobny do uzyskanego przy użyciu obecnie stosowanych antybiotyków, takich jak: cyprofloksacyna, bleomycyna i kloksacylina, obserwowany w testach MIC i MBC. Należy również zauważyć, że koszt otrzymanych związków jest niski, co może być atrakcyjną alternatywą dla obecnie stosowanych środków przeciwdrobnoustrojowych.

Wnioski. Obserwowane wyniki są szczególnie istotne ze względu na rosnącą oporność bakterii na różne leki i antybiotyki.

Introduction

The natural oral microbiome includes 1,200-1,600 bacterial strains, but only 10% of the analyzed strains have been tested for the induction of advanced periodontal diseases (1-4). Pathogenic strains form advanced clusters of the so-called biofilms that group into specific functional groups called complexes, which were well characterized by Sokransky (5). They secrete primary and secondary products of their metabolic processes, endotoxins, exotoxins and proteolytic enzymes, into the oral environment (6-9). And the saliva present in the environment directly affects the periodontal tissues, causing their destruction. This causes the formation of numerous gangrees, swellings, inflammations and canker sores, which in turn leads to spontaneous tooth loss. This condition occurs as a result of the body's imbalance (homeostasis) between positive and negative strains in the oral cavity and the physiology of the mucous membrane and epithelium covering the entire oral cavity – leading to the formation of oxidative stress, which favors the conditions for the multiplication of pathogenic bacterial strains (10-14).

It is also related to the oxidation-reduction potential of a given niche, which creates favorable conditions for the development of pathogenic anaerobic bacteria, which in the form of biofilms use nutrients more effectively and defend themselves more effectively against the body's protective mechanisms (14-16). An example is leukotoxin produced by the red complex bacteria *A. actinomycetemcomitans*, which is responsible for the destruction of neutrophils and monocytes, leading to cell lysis (increasing the permeability of their membranes). Also, endotoxins from other red complex bacteria such as *P. gingivalis* and *A. actinomycetemcomitans* cause the release of substances such as interleukin 1 beta and prostaglandin E2 by monocytes, fibroblasts and macrophages, which actively participate in bone resorption (18-24). Additional building blocks for the life and development of pathogenic microorganisms living in the oral cavity are simple amino acids, proteins, complex carbohydrates and glycoproteins contained in saliva. Fluids flowing from above and subgingival crevices are a very rich source of nutrients for bacteria. Also an endogenous generator of nutrients for bacteria of Sokransky complexes are substances released by the rotting of diseased periodontal tissues under the influence of hydrolytic enzymes, which include: proteases, collagenases, hyaluronidases, DNAases (22-33).

Many enzymes secreted by pathogenic bacteria destroy substances contained in the intercellular matrix

(e.g. collagen) and the connective tissue of the macroorganism. These bacteria also secrete into the intercellular environment metabolic products containing nitrogen, such as ammonia, indole, all types of amines, produced in large quantities, and volatile sulfur compounds that destroy the permeability of the mucous membrane and are factors reducing collagen synthesis (23). The rate of colonization of pathogenic microorganisms in the oral cavity also depends on the availability of individual nutrients necessary for them and on exceeding the natural limit of non-specific immunity. Which causes them to occupy specific ecological niches and multiply rapidly in real time. This allows the penetration of other bacterial species, starting ecological succession, which leads to the creation of a large and very diverse environment of resident pathogenic microflora in the oral cavity, which begins to cause specific pathological changes. Starting from the first erupting tooth to the complete denture. In this way, a new autogenous succession is created (24-33).

The preparations used and the ingredients contained in them based on compounds of chemical origin are a type of so-called peptidomimetics, i.e. substances that have a similar structure to e.g. antibiotics commonly used in periodontal diseases, but perform different functions in the body. These compounds also induce strong oxidative stress, but not in the oral cavity but in the bacteria themselves, destroying the structure of the lipid membrane containing LPS (16-18). Ensuring the development of beneficial bacteria with probiotic properties such as Lactobacillus and Bifidobacterium, but also some yeasts, e.g. *Saccharomyces cerevisiae* or *boulardi*, which have a beneficial effect on the body. Their benefits, however, depend on the size of the doses used, the frequency and regularity of their intake. We should also remember the important role of prebiotics for the oral cavity, such as xylitol (simple sugar), which induces the growth and activity of two species of bacteria important for the health of the host organism – *Bifidobacterium* and *Lactobacillus*.

The exact mechanism of colonization of the oral cavity by bacterial microflora has been extensively explained in the works of Rowińska (19-21).

Maintaining gum health in patients with or undergoing treatment for implants periodontal diseases necessarily involves the use of appropriate mechanical tools good oral hygiene. Conditions after implant rehabilitation and teeth exposed to the effects of periodontitis combine changes in the physiological anatomical structure: recessions, implant prostheses, effects of oral surgery gears, wide interdental spaces, residual pockets. These can affect both biofilm accumulation and the possibility of release bacterial colonization of newly formed dental plaque (23-33). The basic task of a hygienist and dentist is to make a well-thought-out choice the ideal combination of manual tools for a specific clinical situation every patient. Such a set involves the use of special instruments designed to deal with specific, specific clinical problems. Otherwise it is impossible to maintain proper hygiene using, for example, regular toothbrushes. Thanks to the synergy of various ingredients which act as the mentioned peptidomimetics, CURASEPT PREVENT liquid, paste or gel works in a way targeted at all strains defined according to Sokransky's terminology to maintain an ideal oral microbiota. CURASEPT PREVENT inhibits the multiplication of the most aggressive ones microorganisms, restores balance of the cavity microflora oral and improves health gums, even in case risk factors. Curasept Prevent Probiotic is a dietary supplement that is distinguished by a unique combination probiotic bacteria:

- *Bifidobacterium lactis* HN019: is one of the most widely used probiotics in medicine and food. Studies have demonstrated the ability to improve treatment outcomes periodontal care and facilitate health maintenance and treatment results in the long run,
- *Kluyveromyces marxianus fragilis* B0399: has a preventive effect unpleasant breath odor, which is one of the most common symptoms in patients with gum problems.

Curasept Prevent Probiotic is also distinguished by the presence of:

- Colostrum: provides numerous protective substances for the mucous membrane and antibacterial enzymes.
- Biotin or Vitamin B_8: supports proper functioning mucous membranes of the digestive tract. Thanks to its ability to adhere to teeth and oral mucosa, it creates a film protective, which hinders the adhesion and colonization of bacterial plaque and oral biofilm. Prevents also occurrence halitosis. Appropriate combinations of microorganisms, combinations with other substances, doses and the frequency/regularity of taking them can bring real benefits in a wide range of year of oral prophylaxis (fig. 1).

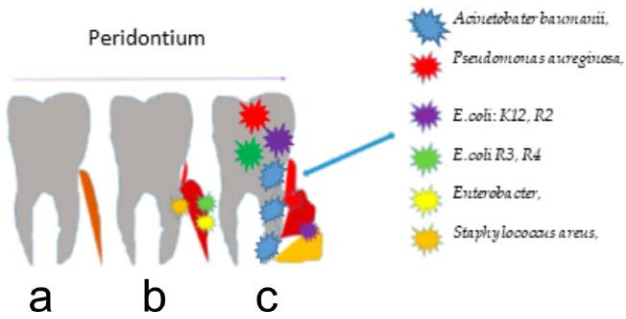

Fig. 1. The process of periodontal disease: a) initial period initiating colonization by bacteria, b) full colonization by bacteria, c) developed periodontal disease

Mainly, the lack of good oral hygiene leads to gingivitis and then to periodontal disease (periodontitis), the above-mentioned condition is associated with discomfort, reduced quality of life and tooth loss. Over time, it contributes to

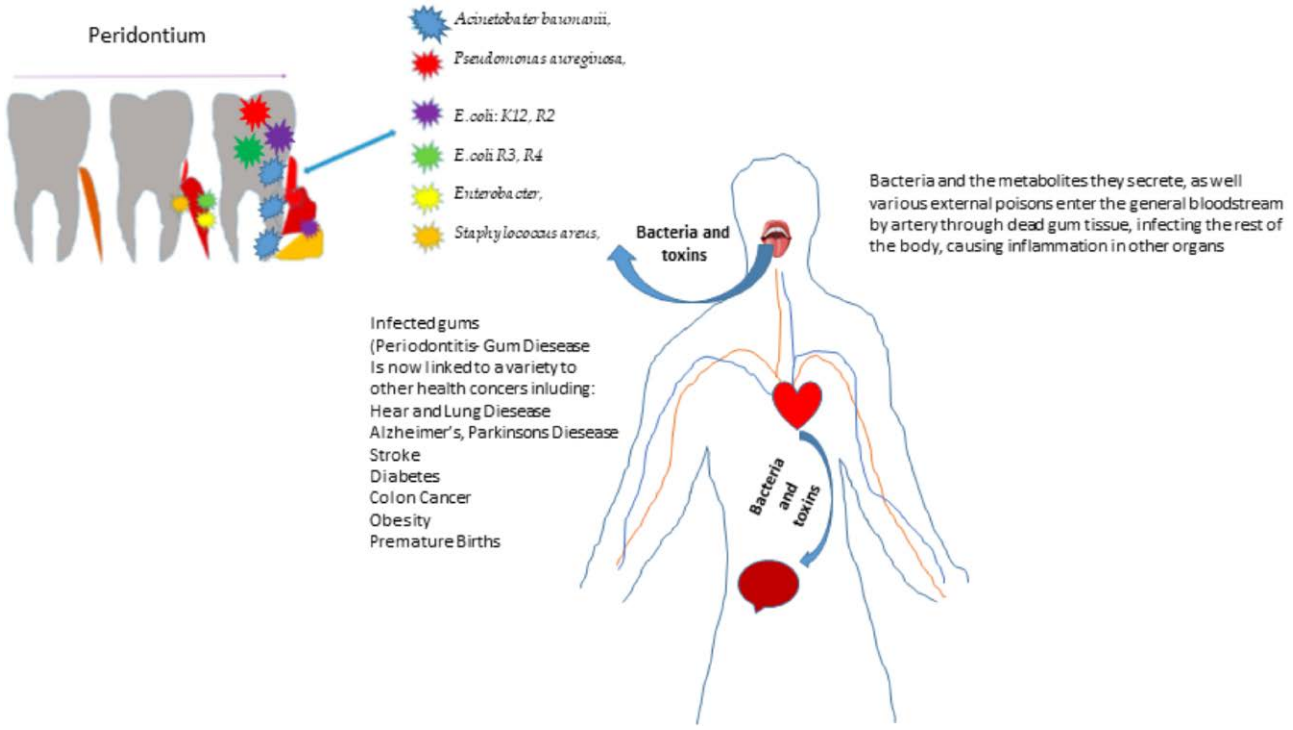

Fig. 2. Circulation bacteria and their toxins inside human body organisms

the generation of systemic diseases, e.g. cardiovascular, diabetes and respiratory diseases (fig. 2).

Aim

The aim of the study was to check the antibacterial properties of mouthwashes recommended by Indent – a leader in dental prophylaxis and distributor of companies such as Curasept, Tello, Frezyderm Curaprox, AloeFresh, which help accelerate healing and prevent persistent infections or recurrent canker sores.

Material and methods

Microorganisms and media

The reference bacterial strains of red complex were provided from (LGC Standards, Manchester, U.K.) and growth media were used as described in Kucia (22). The deposition of tartar as a result of the deposition of unremoved bacterial plaque on the teeth, maintains the inflammation manifested by bleeding gums, swelling and pain. Based on the analyzed indicators, we were able to estimate the state of oral hygiene and its level.

The reference bacterial strains of *E. coli* (K12 ATCC 25404, R2 ATCC 39544, R3 ATCC 11775, R4 ATCC 39543), *Staphylococcus aureus strain* (ATCC 23235), as well as on *Acinetobacter baumannii* (ATCC 17978), *Pseudomonas aeruginosa* (ATCC 15442), *Enterobacter cloacae* (ATCC 49141) were provided from (LGC Standards U.K.) and were used according to the recommendation of ISO 11133: 2014. These strains were used to test antibacterial activity with analyzed compounds by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as described in (8, 9, 24-26) and are show in supplementary materials in figure S1.

Minimum inhibitory concentration (MIC)

The MIC was estimated by a microtiter plate method using sterile 48 or 96-well plates (69-78). 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 10(6) 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.

Statistical analysis

The Statistica program (version 12, StatSoft, Tulsa, OK, USA) was used for the research. The examined characteristics in different groups are presented as mean values, with standard deviation. Results were analyzed with one-way Analysis of Variance (ANOVA). When the F ratio was significant, the Tukey test was used. The level of statistical significance was analyzed at $p < 0.05$.

Analyzed compounds

1. CURASEPT PREVENT PROBIOTICO − probiotic strengthening mucous membranes 14 tablets

Curasept Prevent Probiotico is a dietary supplement that contains a variety of strains selected to support the balance of the oral microbiota and protect the health of the oral mucosa. It is intended for daily use to prevent gingivitis. It is especially recommended for implant patients and high-risk groups, such as smokers, obese people, people with autoimmune diseases, people with recurrent gingivitis, and people with cardiovascular pathologies.

2. Curasept PREVENT preventive toothpaste against gum disease with colostrum, 75 ml

The Curasept PREVENT series was developed to maintain eubiosis and the health of the oral mucosa. An innovative set of selected substances supports the body's natural protective and healing mechanisms occurring in the human oral cavity. Qualitative and quantitative changes in microflora that occur continuously throughout our lives may disturb the bacteriological balance and cause a transition to "dysbiosis", which results in the reproduction of aggressive and undesirable pathogens. This may result in inflammation of the oral cavity leading to the development of tissue disease and periodontitis. The Curasept PREVENT series is designed to strengthen, protect and prevent gum diseases.

Active ingredients of Curasept PREVENT toothpaste:

- ozonated oil − gradually releases ozone and creates a favorable micro-environment for maintaining the balance of oral microflora. Improves the condition of gums,
- colostrum (colostrum) − a natural agent that supports the regeneration and strengthens the gums of the oral cavity thanks to the content of enzymes and proteins with a defensive effect that interact with lactoferrin and lysozyme,
- PVP-VA − thanks to the ability to adhere to teeth and mucous membrane, creates a protective layer, hindering the adhesion and proliferation of bacterial plaque and delays the formation of biofilm,
- tea tree oil − contains a natural set of substances with antioxidant properties. Effectively prevents the deposition of bacterial plaque and inhibits the formation of tartar,
- stevia − a natural sweetener with anti-cariogenic effect. It does not contain calories or gluten.

3. CURASEPT ADS 350 − Gel for local treatment of gums with chlorhexidine, 30 ml

Anti-plaque gel with chlorhexidine 0.5%. Chlorhexidine has excellent antibacterial properties and very high substantivity, thanks to which it adheres perfectly to the teeth and gum mucosa, and is characterized by prolonged action. The special ADS formula prevents brown discoloration on teeth and mucous membranes, even after prolonged use. Its use does not result in taste disorders in patients, which are typical of other chlorhexidine solutions. It does not irritate the oral mucosa.

Advantages of CURASEPT ADS 350 gel for topical use:

- contains 0.50% chlorhexidine diglutanin solution,
- gives a prolonged antiseptic effect,
- fights mouth ulcers,
- stimulates the healing of gums after surgery,
- does not contain SLS, is safe for soft tissues and does not disturb the concentration of chlorhexidine,
- does not disturb the taste,
- the ADS system protects against brown discoloration on the mucous membrane without side effects,
- it can be safely used for two months.

For local treatment of gums:

- contains 0.5% chlorhexidine for use against bacterial plaque,
- protects gums and interdental spaces before and after dental treatment,
- removes dental plaque from the oral cavity before dental procedures and stimulates the healing of gums after surgical procedures,
- prevents the risk of oral infection,
- fights ulcers in the mouth,
- treats gingivitis,
- fights inflammation caused by denture irritation.

Ingredients: Water, Propylene Glycol, Xylitol, Hydroxyethyl Cellulose, Chlorhexidine Digluconate, Ascorbic Amide, Peg-40 Hydrogenated Castor Oil, Sodum Metabisulphite, Aroma, Methylparaben Chlorhexidine (CHX) content 0.5% Volume 30 ml.

4. Curasept soothing periodontal gel with chlorhexidine 0.5% + chlorobutanol + PVP-VA 30 ml

Thanks to the presence of PVP-VA, the gel creates a protective layer on the gums, supporting regeneration, and also soothes the symptoms of irritation of the oral mucosa. It is recommended for quick treatment of mucosa damage after dental procedures. The special ADS formula contained in CURASEPT products significantly reduces the possibility of discoloration on teeth and mucous membranes. Gel composition: Purified Water, Propylene Glycol, Xylitol, Hydroxyethylcellulose, PEG-40 Hydrogeneted Castor Oil, PVP-VA Copolymer, Chlorobutanol, Chlorhexidine Digluconate, Ascorbic Acid, Sodium Metabisulfite, Sodium Citrate, Aroma, Sodium Benzoate, Citric Acid, C.I. 17200.

5. Curasept Gel Toothpaste Intensive Treatment Chlorhexidine 0.20%, 75 ml

6. CURASEPT BIOSMALTO Caries Abrasion & Erosion – paste for intensive prevention of tooth enamel, 75 ml

Intensively remineralizing enamel and dentin toothpaste for daily tooth brushing. The innovative Curasept BIOSMALTO series shows immediate, intensive action towards repairing and protecting enamel damage caused by abrasion and erosion by creating a new mineral phase, which is more resistant than the natural phase. Biosmalto Protection toothpaste protection against caries – contains two functional substances in microcrystalline form:

- FLUORO-HYDROXYAPATITE,
- BIO ACTIV COMPLEX, composed of biomimetic hydroxyapatite replaced by magnesium, strontium and carbonate ions combined with chitosan. The complex action of microcrystals means that BIOSMALTO protection against caries has high biological activity, which rebuilds enamel and dentin. The tooth structure is regenerated and the new hard tissues that are formed are enriched in a new mineral phase in which magnesium, strontium and fluorine ions increase resistance to caries, inflammation and abrasion. Chitosan, a bio-adhesive polymer, prolongs the action of active ions and prevents the deposition of bacterial biofilm.

The paste is especially dedicated to:

- prevention in people at risk of increased tooth decay,
- erosive damage to tooth enamel,
- treatment of the so-called "white spots" of tooth enamel,
- during orthodontic treatment,
- in saliva deficiency,
- with tooth hypersensitivity.

Advantages of Curasept BIOSMALTO PROTECTION paste:

- intensively remineralizes enamel and dentin,
- protects against acid erosion and abrasion,
- prevents tooth hypersensitivity,
- prevents tooth decay and inhibits the formation of dental plaque,
- has low abrasion < 56,
- does not contain SLS,
- does not require rinsing the mouth after brushing teeth,
- contains fluorine ions 1450 ppmF.

7. CURASEPT BIOSMALTO PROTECTION – strawberry mousse 50 ml

Research on the optimal composition and operation of Biosmalto products lasted for 7 years. They were conducted by the largest dental universities. Clinical tests of products guarantee the project, which allowed us to introduce to the market products based on biomimetic, biocompatible and bioactive materials that stimulate the mineralization of enamel and dentin. Two functional substances contained in the Biosmalto liquid trigger the process of creating a new mineral phase, which is more resistant to acid attacks and mechanical damage. Therefore, the substances contained in the mousse increase resistance to caries, inhibit the development of bacterial plaque, and stimulate the process of remineralization of enamel and dentin. It also protects against acid erosion and alleviates the symptoms of dentin hypersensitivity, which results from the presence of strontium salts. The mousse contains 1450 ppm of fluorine.

Active ingredients of CURASEPT BIOSMALTO PROTECTION mousse: Engineered Amorphous Calcium Phosphate (ACP). It is characterized by high reactivity and quickly transforms into hydroxyapatite – a building component of enamel and dentin. Upon contact with saliva, the molecule dissolves immediately, quickly releasing active substances that selectively penetrate damaged areas and quickly remineralize enamel and dentin. Due to its small size, the complex penetrates into and closes the dentinal tubules, significantly reducing tooth hypersensitivity. It protects against acid erosion and alleviates the symptoms of dentin hypersensitivity. The mousse strengthens and remineralizes the tooth surface, releasing calcium and fluorine ions.

The mousse contains 1450 ppm of fluorine. Glycerin, PEG-8, Hydrated Silica, Purified water, Strontium Chloride, Calcium Phosphate Carbonate Citrate Fluoride, Xylitol, Xanthan Gum, PEG-40, Hydrogented Castor Oil, Sodium Hyaluronate, Potassium Acesulfame, Silica, P-Anisic Acid, Benzoic Acid, Aroma, Citric Acid, Sodium Hydroxide, F-ACP Complex.

8. Curasept PREVENT preventive toothpaste against gum disease with colostrum, toothpaste supporting the natural microflora of the oral cavity.

Effectively prevents the deposition of bacterial plaque and inhibits the formation of tartar, stevia – a natural sweetener with anti-cariogenic effect. It does not contain calories or gluten. Indications for using Curasept Prevent toothpaste:

- gum disease and inflammation,
- patients after orthodontic and implantological procedures,
- patients after extensive and/or complex prosthetic rehabilitation treated for periodontal problems in the maintenance phase,
- restoring the balance of bacterial flora,
- improving the condition and health of oral tissues,
- strengthening the gums in autoimmune diseases, such as: Sjogren, diabetes, hypothyroidism,
- strengthening the gums in people with weakened immune defenses,
- strengthening the gums of smokers and people suffering from dry mouth,
- halitosis.

9. CURASEPT Daycare Protection Booster Frozen Mint Toothpaste with a strong mint flavor

Curasept Daycare Protection Booster toothpaste provides longer protection of the oral cavity against plaque-forming bacteria, up to 4 hours. All thanks to the unique CPC-HAP complex (cetylpyridinium chloride and hydroxyapatite). CURASEPT Daycare Protection Booster Frozen Mint Toothpaste with strong mint flavor 75 ml Curasept Daycare Protection Booster toothpaste provides longer protection of the oral cavity against the bacteria responsible for the formation of dental plaque, up to 4 hours. The paste contains the patented CPC-HAP complex (cetylpyridinium chloride and hydroxyapatite) and essential oils. The benefits of using the paste include, above all, effective protection against caries and gum diseases, as well as protection against unpleasant breath odor. The paste has a strongly refreshing mint flavor. The aluminum tube of polish is recyclable. The paste is recommended for use with Curasept Daycare Protection Booster Frozen Mint liquid.

The main ingredients of the paste and their effects:

- The CPC-HAP complex is a unique combination of cetylpyridinium chloride and hydroxyapatite. Biomimetic hydroxyapatite easily combines with the enamel surface and remineralizes it. At the same time, it gradually releases CPC, which provides longer protection of the oral cavity against harmful bacteria. Protection may last up to 4 hours.
- Essential oils (menthol, eucalyptol, thymol, methyl salicylate) help reduce the accumulation of bacteria and the formation of new dental plaque. They work for a longer period of time below and above the gum line. They protect gums against inflammation and combat the causes of bad breath.
- Sodium fluoride (900 ppm F^-) effectively strengthens tooth enamel and prevents caries.

Paste composition Curasept Daycare Protection Booster: Aqua, Sorbitol, Hydrated Silica, Xylitol, Propylene Glycol, Peg-32, Cellulose Gum, Cocamidopropyl Betaine, Hydroxyapatite, Calcium Glycerophosphate, Sodium Fluoride, Cetylpyridinium Chloride, Calcium Pantothenate 1,2 hexanediol, Potassium Nitrate, Cetraria Islandica Extract, Thymol, Elettaria Cardamomum 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, CI 42090, Cinnamal, 900 ppm F-.

10. Gel with chlorhexidine at a concentration of 0.5% for local treatment of wounds and irritations in the oral cavity

Curasept ADS 350 gel is highly effective in the local treatment of wounds caused by dentures, implants and lesions caused by periodontitis. It also accelerates the healing of all wounds after surgery.

11. Curasept after rapid mouthwash

Curasept after rapid gel for canker sores in the mouth 10 ml Curasept After Rapid is an effective gel for canker sores. The unique gel formula has multidirectional effects. The gel relieves pain, has anti-inflammatory properties and accelerates wound healing. It is perfect for painful canker sores of large and small diameter.

12. Curasept after rapid protective gel

It accelerates healing and acts quickly on pain caused by canker sores and viral infections such as cold sores and fungal infections. It helps in the regeneration of gum tissue by increasing the hydration of the mucous membranes. The product is ideal for local treatment of single and small canker sores.

13. Curasept soothing periodontal gel with chlorhexidine 0.5% + chlorobutanol + PVP-VA 30 ml. CURASEPT PERIODONTOLOGICAL SOOTHING GEL

Thanks to the presence of PVP-VA, the gel creates a protective layer on the gums, supporting regeneration, and also soothes the symptoms of irritation of the oral mucosa. It is recommended for quick treatment of mucosa damage after dental procedures. The special ADS formula contained in CURASEPT products significantly reduces the possibility of discoloration on teeth and mucous membranes.

14. Curasept ADS 712, gel toothpaste with chlorhexidine 0.12%

Curasept ADS 712 gel toothpaste with chlorhexidine 0.12% will help you ensure thorough hygiene of the entire oral cavity. Also in the case of difficult access to all interdental spaces in people wearing orthodontic braces or using dental prosthetics. Chlorhexidine paste with an active ingredient concentration of 0.12% is also perfect after oral surgery. It can also be used in the case of the problem of plaque deposition. Chlorhexidine protects teeth and gums against the accumulation of plaque and the harmful effects of bacteria. This ingredient binds to the oral tissue and is then gradually released, providing a long-lasting feeling of cleanliness. Proper protection against dental plaque helps avoid inflammation and gum disease. Its action is supported by xylitol, which inhibits the growth of bacteria and replenishes valuable minerals. The ADS (Anti Discoloration System) formula used in Curasept ADS 712 paste prevents discoloration and does not cause taste disturbances. This makes the paste better absorbable during oral hygiene. The pleasure of use is complemented by the gel form of the paste, and the delicate mint flavor leaves a feeling of freshness. The formula of the paste is gentle on the mucous membrane.

Chemicals

All reagents and the solvents were purchased from Sigma-Aldrich. All solvents were of analytical grade and were used without prior distillation.

Results and discussion

Analysis of bacterial biofilms in search of periopathogens of bacterial complexes

In our study, we analyzed influence of analyzed 1-14 compounds on model bacteria strains like *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterobacter*, *E. coli*. observed very often in mouth using microbiological methods described earlier by Kucia et al. (22) and sequenced by Sanger methods (33). The microbiome growth of the model pathogenic model strains were observed after treatment with analyzed compounds on analyzed example dishes (fig. 3a-d).

Grown fungal colonies (including yeasts) indicate a serious oral cavity infection and insufficient hygiene, therefore only grown bacterial colonies were used for sequencing, in line with the assumptions of the study.

Sequence analysis of bacterial biofilms grown on plates showed mainly red complex bacteria (tab. 1). The obtained results show that the influence of the specific diet and using specific compounds 1-14 diet can stimulate the development of beneficial microorganisms, including, for example, *L. salivarius*, and it can have a bactericidal effect on pathogenic bacteria.

The results obtained from sequencing using the Sanger method (33) with the right sets of primers for the identification of bacterial biofilms from the gingiva surfaces. The values presented in table 1 show identifications at the level of 99.99-100% of the analyzed species or strains of pathogenic bacteria of the consecutive six complexes (tab. 1). We could observe the presence of bacteria from each of the 6 bacterial complexes of which the largest share is the bacteria found in complexes: orange > red > yellow > green ≥ violet > blue where the reaction equilibrium was shifted towards a specific pH. Bacteria from the six groups were now being recruited. A specific diet regulates the pH of our oral mucosa and changes the niche of bacterial biofilms towards beneficial bacteria and not the pathogenic bacteria that induce tooth decay (fig. 4).

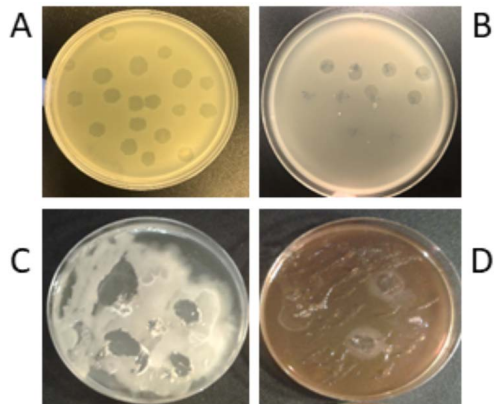

Fig. 3a-d. Example of dishes after treatment of analyzed first 4 of all analyzed 14 compounds: *Acinetobater baumannii* (A), P*seudomonas aureginosa* (B), *Stapahylococcus areus* (C), *Enterobacter* (D)

Tab. 1. Sanger sequencing of bacterial inoculum from specific Petri dishes

No	Type	No	Type	No	Type	No	Type
1a	*Propionibacterium acnes*	1b	*Arachnia propionica* (*Actinomyces propionicus*)	1c	*Bifidobacterium dentium*	1d	*Eubakterium timidum*
2a	*Streptococcus constellatus*	2b	*Streptococcus oralis*	2c	*Streptococcus mitis*	2d	*Prevotella nigrescens*
3a	*Streptococcus pygenes*	3b	*Streptococcus mutans*	3c	*Streptococcus sanguinis*	3d	*Streptococcus gordonii*
4a	*Capnocytophaga ochracea*	4b	*Capnocytophaga sputigena*	4c	*Capylobacter concisus*	4d	*Capnocytophaga ochracea*
5a	*Rothia dentocariosa*	5b	*Rothia dentocariosa*	5c	*Trichomonas tenax*	5d	*Treponema denticola*
6a	*Lactobacillus acidophilus*	6b	*Lactobacillus buchneri*	6c	*Bifidobacterium dentium*	6d	*Bifidobacterium dentium*
7a	*Lactobacillus casei*	7b	*Lactobacillus plantarum*	7c	*Lactobacillus fermentum*	7d	*Lactobacillus salivarius*
8a	*Streptococcus sanguinis*	8b	*Peptostreptococcus micros*	8c	*Peptostreptococcus micros*	8d	*Streptococcus gordoni*
9a	*E. coli* R2	9b	*Lactobacillus buchneri*	9c	*Bifidobacterium dentium*	9d	*Lactobacillus casei*
10a	*E. coli* R4	10b	*E. coli* R4	10c	*Lactobacillus casei*	10d	*Lactobacillus plantarum*
11a	*E. coli* R3	11b	*Propionibacterium acnes*	11c	*Propionibacterium acnes*	11d	*Desulfococcus oleovorans* Hxd3
12a	*Tanarella forsythia*	12b	*Tanarella forsythia*	12c	*Tanarella forsythia*	12d	*Tanarella forsythia*
13a	*E. coli* R2	13b	*E. coli* R2	13c	*E. coli* R2	13d	*Eubacterium nodatum*
14a	*Pyrococcus* sp. OT3	14b	*Pyrococcus* sp. OT3	14c	*Pyrococcus* sp. OT3	14d	*Pyrococcus* sp. OT3

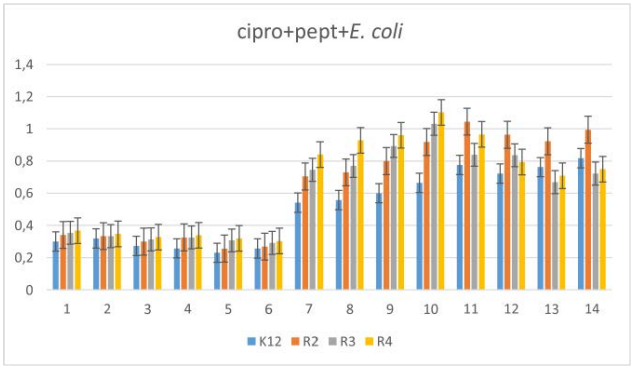

Fig. 4. Minimum inhibitory concentration (MIC) of the 1-14 compounds in model *E. coli* bacterial strains with ciprofloxacin. The x-axis features compounds 1-14 used sequentially. The y-axis shows the MIC value in μg/mL^{-1}. Investigated strains of *E. coli* K12 as control (first on the flot), R2 strains (second on the flot), R3 strain (third on the flot), and R4 strain (fourth on the flot). The y-axis shows the MBC value in μg/mL^{-1}. The order in which the compounds were applied to the plate are shown in Supplementary Materials Figure S1

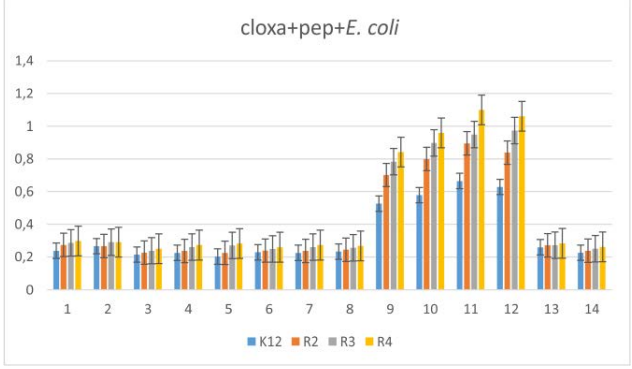

Fig. 5. Minimum inhibitory concentration (MIC) of the 1-14 compounds in model *E. coli* bacterial strains with cloxacillin. The x-axis features compounds 1-14 used sequentially. The y-axis shows the MIC value in μg/mL^{-1}. Investigated strains of *E. coli* K12 as control (first on the flot), R2 strains (second on the flot), R3 strain (third on the flot), and R4 strain (fourth on the flot). The y-axis shows the MBC value in μg/mL^{-1}. The order in which the compounds were applied to the plate are shown in Supplementary Materials Figure S1

Model strains of *E. coli* were plotted in all 48-well plates observed; K12, R2-R4 which were treated with the analyzed compounds. From analysis of the MIC assays, color changes were observed for all compounds tested, but at different levels and at different dilutions. Bacterial strains R3 and R4 were the most susceptible to modification with these compounds due to the increasing length of their LPS (visible dilutions of 10-3 corresponding to a concentration of 0.25 μM) than strains K12 and R2 (visible dilutions of 10(-6) corresponding to a concentration of 0.06 μM). The analyzed R4 strain was the most sensitive of all strains, probably due to the longest length of the lipopolysaccharide chain in the bacterial membrane. The same effect was observed by clinical model strains (tab. 2).

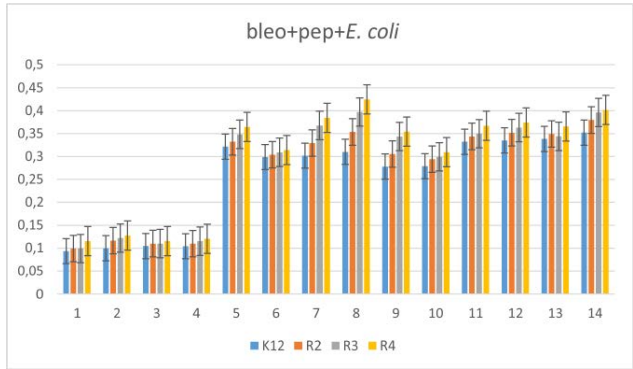

Fig. 6. Minimum inhibitory concentration (MIC) of the 1-14 compounds in model *E. coli* bacterial strains with bleomycin. The x-axis features compounds 1-14 used sequentially. The y-axis shows the MIC value in μg/mL^{-1}. Investigated strains of *E. coli* K12 as control (first on the flot), R2 strains (second on the flot), R3 strain (third on the flot), and R4 strain (fourth on the flot). The y-axis shows the MBC value in μg/mL^{-1}. The order in which the compounds were applied to the plate are shown in Supplementary Materials Figure S1

Tab. 2. Statistical analysis of all analyzed compounds by MIC, < 0.05*, < 0.01**, < 0.001***

No. of samples	1,2,3,4	5,6,7,8	9-14	Type of Test
K12 E. coli	**	**	**	MIC
R2 E. coli	**	**	**	MIC
R3 E. coli	**	**	**	MIC
R4 E. coli	**	**	**	MIC
Acinetobacter baumannii	**	**	***	MIC
Pseudomonas aeruginosa	**	**	***	MIC
Enterobacter	**	**	***	MIC
Staphylococcus aureus	**	**	***	MIC
ciprofloxacin	***	***	*	MIC
cloxacillin	***	***	*	MIC
bleomycin	***	***	*	MIC

The analysis of clinical bacterial strains modified with tested compounds 1-14

The obtained MIC values, as well as our previous studies with various types of the analyzed compounds indicate the analyzed 1-14 compounds with specific components show a strong toxic effect on the analyzed bacterial model strains. Performed studies proved that the analyzed and newly used 1-14 compounds can potentially be used as "substitutes" for the currently used antibiotics in hospital and clinical infections by analyzed clinical strains such as: *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, Enterobacter, *Staphylococcus aureus* (fig. 5-9).

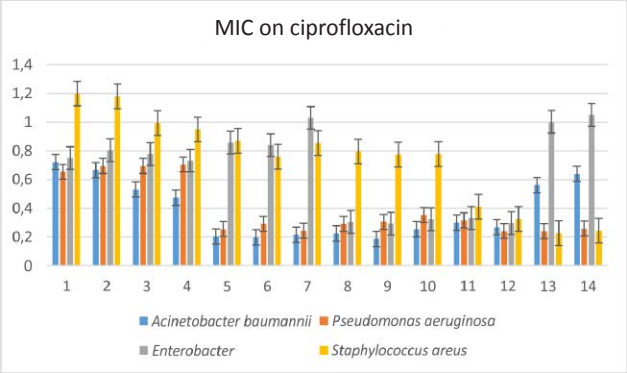

Fig. 7. Examples of MIC with model bacterial clinical strains using antibiotics ciprofloxacin (cipro), The x-axis features antibiotics used sequentially. The y-axis features the MIC value in $\mu g/mL^{-1}$. The order in which the compounds were applied to the plate are shown in Supplementary Materials Figure S1

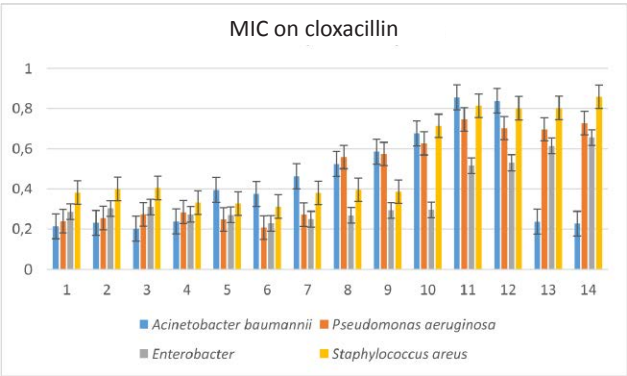

Fig. 8. Examples of MIC with model bacterial clinical strains using antibiotics cloxacillin (clox), The x-axis features antibiotics used sequentially. The y-axis features the MIC value in $\mu g/mL^{-1}$. The order in which the compounds were applied to the plate are shown in Supplementary Materials Figure S1

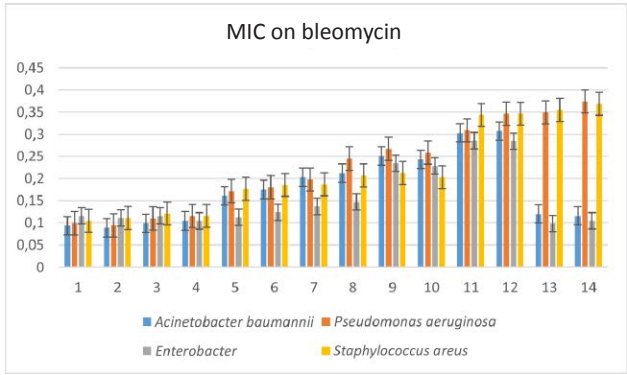

Fig. 9. Examples of MIC with model bacterial clinical strains using antibiotics bleomycin (bleo), The x-axis features antibiotics used sequentially. The y-axis features the MIC value in $\mu g/mL^{-1}$. The order in which the compounds were applied to the plate are shown in Supplementary Materials Figure S1

The synthesis of new compounds from 1-14 Curasept family, similarly to other compounds studied by us in previous works, may constitute a new alternative to the commonly used antibiotics in clinical infections. Therefore, the analyzes of new compounds from this group are extremely important in nosocomial or clinical infections. The analyzed compounds were tested for their toxic effect on cells of *Escherichia coli* K12 strains (having native LPS) and R2-R4 (with different LPS lengths). On the basis of the observed results in the MIC and MBC tests, it was found that the analyzed compounds significantly influenced the defragmentation of the membrane and the structure of the cell wall of bacteria containing LPS of various lengths, causing oxidative stress caused by (modification) of the analyzed compounds to damage and modification of bacterial DNA. The presented research shows that 1-14 will be used in the future as typical "substitutes" for new drugs in relation to the antibiotics used in hospital infections. This chemical and biological activity is related to specific substituents in their structure (tab. 1). The obtained MIC values, as well as our previous studies with various types of the analyzed compoundsindicate that also 1-14 compounds show a strong cytotoxic effect of the analyzed model bacterial strains K12 and R2-R4 and model clinical strains , due to the type and kind of group at *para*-position.

Probably the length of the alkyl chain and the type of the specific substituent may determine the toxicity of the analyzed *E. coli* strains, including in particular R4, as evidenced by the obtained MIC values. Modifications with antibiotics were smaller and not as clear as in the case of the analyzed compounds (see Supplementary materials fig. S1). The destabilization of the inside bacterial complex that regulates these enzymes is possibly crucial for the survival of bacterial cells and may play an important role in changing its electrokinetic potential expressing the reversal of burdens. Blocking these enzymes by 1-14 compounds of Curasept family, stops DNA replication causing bacterial cells to apoptosis and be destroyed. In the future, cytotoxicity studies will also be carried out using various cell lines and cultures to assess the biocompatibility of test compounds with active 1-14 compounds. Dysfunction of bacterial membranes containing different lengths of LPS in model bacterial strains is an ideal model to assess the effectiveness of these compounds in relation to the antibiotics used.

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. Based on specific periodontological indicators, the dental community, including doctors, dentists, and hygienists, will be able to estimate with 100 percent certainty when and at what time, based on the data obtained from the indicators, a bacterial biofilm will be formed and what antibiotic therapy should be applied. 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

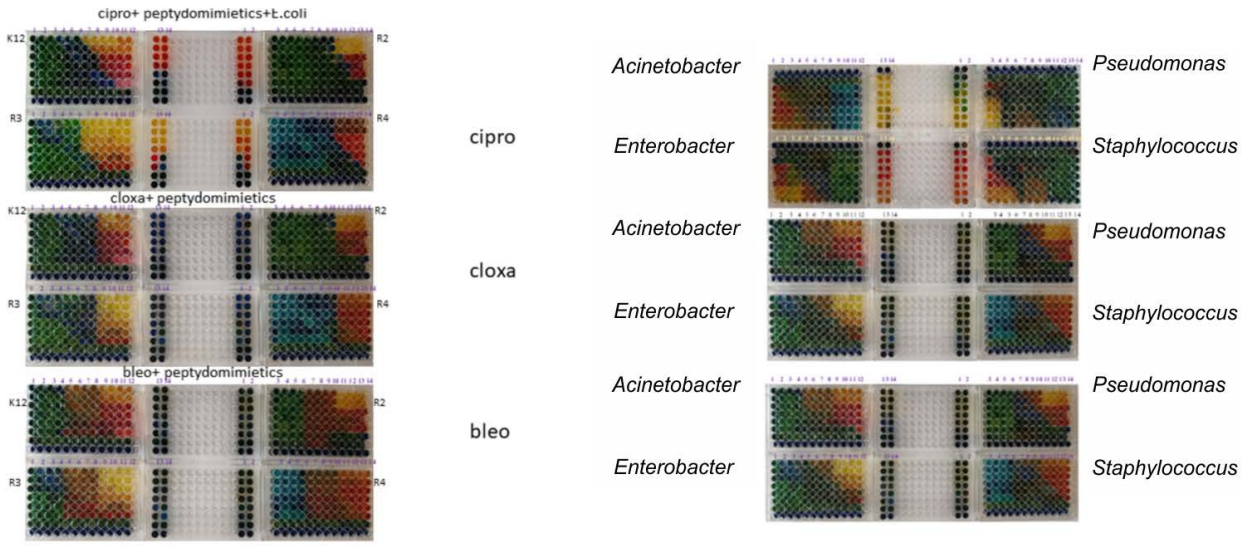

Fig. S1. Examples of MIC on microplates with different concentration of studied compounds (μg/mL^{-1}). Resazurin was added as an indicator of microbial growth with model K12, R2, R3, and R4 strains and clinical strains such as: *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter*, *Staphylococcus aureus* with tested compounds, as described in figures 4 and 5. Additionally, examples of MIC with different strains K12, R2, R3, and R4 of studied antibiotics with ciprofloxacin (ci), bleomycin (b), and cloxacillin (cl) in (μg/mL^{-1})

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 recommendations issued by the American Dental Association indicate that systemic antibacterial drugs should be used with rational dosing to avoid side effects affecting the body (17, 23). In addition to vaccines based on COVID-19, the search for new methods enabling faster detection of bacterial biofilms and correlation with the function of a specific diet will allow appropriately targeted clinical and diagnostic tests to be conducted. Our research has shown that the effects of 14 different compounds depending on the sensitivity of the host (11, 12) (tab. 1). In fact, the levels of the analyzed pathogens obtained by Sanger sequencing (33) of bacteria (tab. 1). The inhibition of growth by bacteria of the fifth complex in oral colonization is critical because these bacteria may indirectly influence neoplastic diseases related to pancreatic cancer and cardiovascular diseases. Therefore, the use of a safe traditional diet gives a better effect than any type of food additives or spoilers for determining the effect of pathogenic microorganisms in the diet function and periodontal inflammation. Hygiene determines the health of the oral cavity, and deviations from the accepted norms are a harbinger of upcoming health problems, or the determination of already existing pathological conditions. 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. Based on specific periodontological indicators, the dental community, including doctors, dentists, and hygienists, will be able to estimate with 100 percent certainty when and at what time, based on the data obtained from the indicators, a bacterial biofilm will be formed and what antibiotic therapy should be applied. 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 applied diet does not exclude the use of its various supplements, which can potentially contribute to a significant improvement in the soft tissues of the periodontium, as described in our previous work (23-32). However, a analysed 1-14 compounds rich in macro and microelements and specially usefull surfactants without a significant contribution of carbohydrates and fats significantly contributes to the quality and improvement of vitality and health condition of the periodontium as well as of the teeth themselves, protecting them against early and rapid loss. Based on the obtained results we can state that a specific diet determines the selection of a specific microbiota of the organism in our mouth and the advantage of beneficial bacteria over pathogens from the 6 analyzed complexes. The obtained results suggest that the substances contained in the 1-14

analyzed compounds, may also interact with red complexes of bacteria showing cariogenic activity present in the human oral cavity (11, 12). Chronic inflammation in the oral cavity may lead to the destruction of the alveolar bone, which may consequently lead to tooth loss. This process may involve direct damage to tissues by the secreted toxins of pathogenic bacteria. Recent literature data indicate the effect of bacterial biofilm on various organs and systems of a person, including the nervous system, contributing to the formation of neurodegenerative diseases (19). Currently, antibiotic resistance among pathogenic bacteria is becoming more and more common, leading to super-resistance. The recommendations issued by the American Dental Association indicate that systemic antibacterial drugs should be used with rational dosing to avoid side effects affecting the body (17, 23). In addition to vaccines based on COVID-19, the search for new methods enabling faster detection of bacterial biofilms and correlation with the function of a specific diet will allow appropriately targeted clinical and diagnostic tests to be conducted. The analyzed pastes, gels and rinses are extremely effective and have a very high antimicrobial potential.

Conclusions

The newly analysed compounds were tested as potential antimicrobial agents and the impact of their structure on the antimicrobial activity against model strains of selected clinical strains described by Socransky's. 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.

Conflict of interest
Konflikt interesów

None
Brak konfliktu interesów

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