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# Antibacterial activity of selected commercial products for mouth washing and disinfection, assessed in accordance with PN-EN 1040

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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**Background:** Currently, there is a wide range of products for mouth washing on the Polish market. They have different qualitative and quantitative compositions, and they differ particularly in the concentration of active substances. In antisepsis and disinfection, the significant reduction in number of cells of microorganisms in a particular environment is very crucial. The chemical agents should provide a significant decrease in number of microorganisms in a relatively short time. The purpose of this study was to examine the bactericidal activity of selected herbal products used for treatment of inflammation, and disinfection and washing of the mouth, having antibacterial activity as declared by the manufacturers.

**Material/Methods:** The study included 28 products for mouth washing and disinfection available in Poland. Bactericidal activity was studied using a quantitative suspension test according to the standard PN-EN 1040.

**Results:** Only 1 of 4 tested herbal products, registered as medicinal products, showed satisfactory antibacterial activity when they were used according to the manufacturer's recommendations. A total of 13 preparations (48%) complied with the standard requirements against all tested strains. Up to 19% of products showed no bactericidal activity against bacterial strains, and up to 33% were only effective against certain microorganisms.

**Conclusions:** The informational literature accompanying most antiseptics should be corrected by the manufacturers, providing information about antimicrobial activity consistent with the requirements of applicable standards. The information on the packaging or in the leaflets for antiseptic products should be corrected by the manufacturers to include accurate information on antimicrobial activity.

**Key words:** antimicrobial activity • antiseptics • antisepsis • disinfection • products for mouth washing and disinfection

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## Background

In the Polish market there is currently a broad range of products for mouth washing and disinfection. These products differ in qualitative and quantitative composition and in the concentration of active substances. In antisepsis and disinfection, the significant reduction in number of microorganism cells in a particular environment is crucial. The chemical agents should provide a significant degree of microorganism reduction in a relatively short time period.

The European Committee for Standardization (CEN) has been developing standards for evaluation of the effectiveness of antiseptics and disinfectants for many years. These standards allow standardization of analytical methods used in tests. The relevant EN standards (currently adopted as the Polish standards PN) allow comparison of antimicrobial activity of a number of products with the same application. Previously, this comparison was impossible due to the use of various, diverse procedures (different contact time, concentrations, and tested strains).

The purpose of this study was to examine the bactericidal activity of selected herbal products and other products for mouth washing, possessing antibacterial activity as declared by the manufacturers, used in treatment of inflammation and for disinfection of the mouth.

## Material and Methods

A total of 28 products for mouth washing and disinfection, available in department stores and pharmacies, were included in this study. Their characteristics are presented in Table 1. Bactericidal activity was studied according to the PN-EN 1040 standard "Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics - Test method and requirements (Phase 1)" [1]. It is assumed that the product possesses antibacterial properties if it causes minimum of 5 log reduction in the number of viable bacteria after 60 min or less, when the test organisms are *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442. Additionally, 2 other bacterial strains were included in the study: *Escherichia coli* NCTC 10538 and *Enterococcus hirae* ATCC 10541. These strains are recommended by standard EN 13727 "Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity in human medicine – Test method and requirements (Phase 2/Step 1)" [2].

To perform the test, the densities of bacterial suspensions were adjusted to a value of $1.5–5\times10^8$ cfu/mL. Two contact times were used: 5 min and/or 60 min. At the end of the contact time, bactericidal activity was immediately neutralized by using neutralizer consisting of lecithin (AppliChem) – 3 g/L, Polysorbate 80 (POCH Gliwice) – 30 g/L, sodium thiosulfate (Sigma) – 5 g/L, L-histidine (Merck) – 1 g/L, saponin (Sigma) – 30 g/L, tryptone (Difco) – 1 g/L, NaCl (POCH Gliwice) – 8.5 g/L, and water – up to 1 L.

During this study, the dilution-neutralization method was applied. The test procedure was as follows: 1 mL of water and 1 mL of bacterial test suspension were added to 8 mL of the examined product test solution. At the end of the contact time, 5 or 60 min ±10 s, a 1-mL sample of the test mixture was transferred into a tube with 8 mL of neutralizer mixed with 1 mL of water. After 5 min ±10 s of neutralization time, a 1-mL sample of neutralized test mixture was immediately taken in duplicate and inoculated using the pour plate technique. The plates were incubated in 37±1°C. After 24 h, the number of colony-forming units (cfu) was determined. After 48 h of incubation, verification of the absence of toxicity of the neutralizer and validation of the dilution-neutralization method tests were performed at the same time and under the same conditions. Tests of antibacterial activity of products were performed in duplicate.

## Results

An assessment of the effectiveness of the bactericidal activity in 2 contact times (5 and 60 min), was carried out on 4 herbal products listed in the Register of Medicinal Products authorized in Poland: Dentosept A, Salviae, Stomatosol, and Salviasept.

After conducting a preliminary study of the efficacy of Dentosept A, which was not diluted (concentration recommended by the manufacturer), a reduction in the number of bacterial cells within all tested strains required by the standard was noticed. In the case of 70% solution of this product, reduction in number of bacterial cells of 4 tested strains was about 5 log. However, this result occurred for *E. hirae* after 5 min, but took over 60 min for other strains.

The bactericidal efficacy of the Salviae product based, on the preliminary studies, was insufficient at the concentration recommended by the manufacturer (approximately 2%). In accordance with PN-EN 1040, the product Salviae possesses bactericidal activity for all tested strains at a concentration of 30%. The analysis of the bactericidal activity of the product showed that a 10% solution was effective only against *E. coli* after 60 min. A 20% concentration of this product reduced the number of viable bacterial cells of *S. aureus* after 60 min.

Tests of the effectiveness of the Salviasept product showed that, in the dilution recommended by the manufacturer (1.6%), it meets the requirements of the PN-EN 1040 standard after





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**Table 1.** Detail information of analyzed products for mouth rinsing and disinfection analyzed in this study.

| No | Name of the product | Manufacturer | Composition |
|---|---|---|---|
| 1 | Corsodyl* | SmithKline Beecham | Chlorhexidine gluconate, water |
| 2 | Dentosept A* | PhytoPharm Keka S.A. | Extractum fluidum (0.65:1) ex:<br>Matricariae flos, Quercus cortex, Salviae folium, Arnica herba, Calami rhizoma, Menthae piperitae herba, Thymi herba Anaesthesium, Natrium tetraboricum<br>Excipients:<br>Ethanol [32–37% (V/V)], Glycerine, Methylcellulose, Purified water |
| 3 | Hascosept* | Hasco-Lek S.A. | Benzydamine hydrochloride, glycerol, ethanol, saccharin, sodium bicarbonate, methyl p-hydroxybenzoate E 214, mint flavor, polysorbate 20, quinoline yellow, patent blue, purified water |
| 4 | Salviae* | Phyto Pharm | Salviae tinctura (1:5)<br>Excipient: Ethanol [60–70% (V/V)] |
| 5 | Salviasept* | Herbapol Lublin S.A. | Salviae oleum, Thymi oleum, Majoranae oleum, Cineol, Menthae pip. Oleum, Caryophylli oleum, Menthol<br>Extr. fl 1:3.3<br>Ex Chamomillae anth., Salviae fol., Millefolii herba, Menthae pip. Herba, Thymi herba, Foeniculi fruct.<br>Ethanol [52%] |
| 6 | Stomatosol* | Elanda | Gallae tinctura, Arnica tinctura, Tormentilla rhizoma tinctura, ethanol (60–70%) |
| 7 | Active Oral Care Extra Strength Original | Drammock International | Alcohol, glycerin, polysorbate 20, fragrance, sodium benzoate, cetylpyridine chloride, sodium fluoride, sodium saccharin, benzoic acid, allura red, yellow dye, water |
| 8 | Aquafresh** | Glaxo SmithKline | Alcohol, fragrances, cocamidopropyl betaine, sodium saccharin, cetylpyrydine chloride, sodium fluoride, PEG-60-hydrogenated castor oil, sodium bicarbonate, patent blue, chlorophyll, water |
| 9 | Cepacol Antibacterial Mouthwash with Ceepryn | Combe Incorporated | 14% denaturated alcohol, glycerin, fragrances, disodium phosphate, cetylpyridine chloride, polysorbate 80, saccharin, sodium orthophosphate, disodium ethylenediaminetetraacetate |
| 10 | Denivit** | Schwarzkopf & Henkel | Alcohol, xylitol, sorbitol, PEG-60-hydrogenated castor oil, fragrances, sodium fluoride, chlorhexidine gluconate, sodium saccharin, brilliant blue, water |
| 11 | Elgydium** | Pierre Fabre | Glycerin, PEG-40-hydrated castor oil, xylitol, PEG-12-dimethicone, fragrances, hydrofluoride 3-piridinomethanol, potassium sorbate, sodium saccharin, chlorhexidine digluconate, quinoline yellow, patent blue, water |
| 12 | Elmex** | GABA International | PEG-40-hydrogenated castor oil, olaflur (amine fluoride), fragrances, acesulfame potassium, sodium fluoride, poliaminopropyl biguanide, hydrochloric acid, water |
| 13 | Eludril** | Pierre Fabre | Glycerin, alcohol, water, fragrances, cochineal red, chlorhexidine digluconate, chlorobutanol, sodium dietyloheksylosulfosuccinate, limonen, menthol |
| 14 | Mouthwash antiseptic | (produced in the Netherlands, distributor Rossmann) | Alcohol (27.3%), fragrances, PEG-40-hydrogenated castor oil, sodium methylparaben, citric acid, sodium saccharin, tartrazine, cochineal red, sunset yellow, black diamond, water |
| 15 | Mouthwash with fluoride** | (produced in the Netherlands, distributor Rossmann) | Glycerin, PEG-40 hydrogenated castor oil, sodium citrate, imidourea, fragrances, sodium methylparaben, sodium fluoride, sodium saccharin, 2-bromo-2-nitropropano-1,3-diol, brilliant blue, red dye 33, water |
| 16 | Lacalut aktiv** | Arcam GmbH | Glycerin, kokoamidopropyl betaine, PEG-40-hydrated castor oil, propylene glycol, chlorhexidine digluconate, fragrances, aluminum lactogluconate, sodium fluoride, azorubine, water |





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**Table 1 continued.** Detail information of analyzed products for mouth rinsing and disinfection analyzed in this study.

| No | Name of the product | Manufacturer | Composition |
|---|---|---|---|
| 17 | Listerine Cleanmint** | Pfizer | Alcohol, sorbitol, fragrances, poloxamer 407, benzoic acid, sodium saccharin, eucalyptol, zinc chloride, methyl salicylate, thymol, sodium benzoate, menthol, brilliant blue, water |
| 18 | Listerine Freshmint** | Pfizer | Alcohol, sorbitol, fragrances, poloxamer 407, benzoic acid, sodium saccharin, eucalyptol, methyl salicylate, thymol, menthol, sodium benzoate, sodium fluoride, green dye, quinoline yellow, water, |
| 19 | Meridol** | GABA International | Xylitol, crospovidone, PEG-40-hydrogenated castor oil, olaflur (amine fluoride), fragrances, stannous fluoride, sodium saccharine, patent blue, water, |
| 20 | Octenidol | Schulke & Mayr GmbH | PEG-40-hydrogenated castor oil, glycerin, fragrance, sodium gluconate, trichlorosucrose, octenidine hydrochloride, citric acid, BHT, water |
| 21 | Oral B Advantage Mouth Rinse** | Procter & Gamble | Sodium fluoride, cetylpiridine chloride |
| 22 | Oreksyd** | Warszawskie Zaklady Farmaceutyczne Polfa S.A. | Chlorhexidine digluconate, polysorbate 20, sodium saccharin, sodium cyclamate, fragrances, water |
| 23 | Orthokin** | Laboratorios KIN | Sorbitol, glycerin, propylene glycol, PEG-40-hydrogenated castor oil, zinc acetate, fragrances, chlorhexidine gluconate, sodium saccharin, sodium fluoride, menthol, citric acid, cinnamal, D-limonene, water |
| 24 | Paroplak** | Sanofi-Synthelabo | Alcohol, glycerin, PEG-40-hydrogenated castor oil, polysorbate 20, chlorhexidine gluconate, sodium lauryl sulphate, fragrances, methylparaben, sodium fluoride, sodium saccharin, eugenol, allura red, quinoline yellow, water |
| 25 | Pearl Drops** | Church & Dwight UK Ltd. | Citric acid, PEG-40, sodium fluoride, alcohol, saccharin sodium, sorbitol, fragrances, cetylpirydyny chloride, sodium lauryl sarcosinate, sodium citrate, water |
| 26 | Protefix Dental Antisept ** | Queisser Pharma GmbH | Sodium acetate, acetic acid, sorbitol, alcohol, sorbitan oleate, colorant E 122, fragrances, chlorhexidine gluconate, water |
| 27 | Sensikin** | Laboratorios KIN | Sorbitol, glycerin, potassium nitrate, PEG-40 hydrogenated castor oil, dexpanthenol, sodium fluoride, fragrances, tocopheryl acetate, sodium methylparaben, ethylparaben sodium, propylparaben sodium, sodium saccharin, citric acid, water |
| 28 | Walgreens Fresh Breath Antiseptic Mouth Rinse | Walgreen Co. | Eucalyptol, menthol, methyl salicylate, thymol, water, alcohol, sorbitol, fragrances, poloxamer 407, benzoic acid, sodium benzoate, cochineal extract |

* Medicinal products listed in the Register of Medicinal Products authorized in Poland; ** cosmetic products listed in the Central Register of Cosmetic Products conducted by KSIoK (National Register Informing about Cosmetics).

5 min only for the *E. coli* strain. However, undiluted product caused a 5 log reduction of all tested bacteria after just 5 min of contact. The antibacterial activity of the product was tested in concentrations of 10%, 20% and 30%. In the case of *S. aureus*, a 20% concentration of Salviasept had bactericidal activity in accordance with PN-EN 1040 after 5 min, but a 10% solution required a 60 min of contact time. In the case of *E. hirae,* this product, at a 10% concentration, complied with the standard only after 60 min contact time and after 5 min at a concentration of 20%. In the *P. aeruginosa* strain, a 10% product solution caused the required reduction in the number of bacterial cells after 5 min contact time.

Preliminary investigations of the Stomatosol product for mouth washing showed that this product, in concentrations recommended by the manufacturer (approx. 4%), did not possess bactericidal activity meeting the standard, but when the product was used in undiluted form, the requirements of PN-EN 1040 were met after just 5 min. Analysis of the bactericidal activity of a 10% concentration of Stomatosol showed a reduction in the number of viable cells of *S. aureus, E. coli,* and *P. aeruginosa* to the level of 5 log after 60 min contact time. In the case of the *E. hirae* strain, a 20% Stomatosol solution showed appropriate activity after 60 min. Five min of contact time was sufficient at the concentration of 20% for the *E. coli* strain only.





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**Table 2.** Bactericidal activity of herbal medicinal products listed in the Register of Medicinal Products.

| Product/concentration | *S. aureus* ATCC 6538 | | *E. hirae* ATCC 10541 | | *E. coli* NCTC 10538 | | *P. aeruginosa* ATCC 15442 | |
|---|---|---|---|---|---|---|---|---|
| | 5 min | 60 min | 5 min | 60 min | 5 min | 60 min | 5 min | 60 min |
| Dentosept A | | | | | | | | |
| **80%*** | >5.27 | >5.27 | >5.09 | >5.09 | >5.04 | >5.04 | >5.29 | >5.29 |
| 70% | <3.90 | >5.27 | >5.09 | >5.09 | <3.67 | >5.04 | <3.92 | >5.29 |
| Salviae | | | | | | | | |
| **~2%** | <3.90 | <3.90 | <3.72 | <3.72 | <3.67 | <3.67 | <3.92 | <3.92 |
| 10% | <3.90 | 4.90 | <3.72 | <3.72 | <3.67 | >5.04 | <3.92 | <3.92 |
| 20% | <3.90 | >5.27 | <3.72 | 4.36 | <3.67 | >5.04 | <3.92 | <3.92 |
| 30% | >5.27 | >5.27 | <3.72 | >5.09 | 4.50 | >5.04 | <3.92 | >5.29 |
| 40% | >5.27 | >5.27 | >5.09 | >5.09 | >5.04 | >5.04 | >5.29 | >5.29 |
| Salviasept | | | | | | | | |
| ~1.6% | <3.90 | <3.90 | <3.72 | <3.72 | >5.04 | >5.04 | <3.92 | 4.26 |
| 10% | 4.59 | >5.27 | 4.50 | >5.09 | >5.04 | >5.04 | >5.29 | >5.29 |
| 20% | >5.27 | >5.27 | >5.09 | >5.09 | >5.04 | >5.04 | >5.29 | >5.29 |
| Stomatosol | | | | | | | | |
| **~4%** | <3.90 | <3.90 | <3.72 | <3.72 | <3.67 | <3.67 | <3.92 | <3.92 |
| 10% | <3.90 | >5.27 | <3.72 | 3.77 | <3.67 | >5.04 | <3.92 | >5.29 |
| 20% | <3.90 | >5.27 | <3.72 | >5.09 | >5.04 | >5.04 | <3.92 | >5.29 |

In bold – concentration recommended by manufacturers; * concentration recommended by manufacturers =100%, however for the examination sample is diluted to 80%.

Higher concentrations of this product were turbid and not homogeneous, making it impossible to carry out further investigations. Detailed results of these products are shown in Table 2.

Due to the presence of ethanol in the tested herbal products, the antibacterial activity of ethyl alcohol at concentrations of 10–50% was conducted on 4 bacterial strains at 2 contact times: 5 and 60 min. A 5 log reduction in the number of bacterial cells was achieved for all tested strains after 5 min contact time for 50% ethanol. Ethanol at a 40% concentration only completely eliminated *S. aureus* cells after 5 min contact time. For *E. coli* and *P. aeruginosa,* this level was achieved after 60 min contact time. The 5 log reduction of *E. hirae* cells was not achieved in any tested concentration.

It was observed that ethanol concentrations of 30% and lower did not show any bactericidal activity against the tested strains. The survey revealed that antimicrobial activity of ethanol can be excluded in the Stomatosol, Salviae, and Salviasept products at concentrations of 50% and lower.

Two other products from the group of medicinal products – Corsodyl and Hascosept – at the level of 5 log bactericidal activity against all tested strains after 5 min of contact time. Undiluted products were tested according to manufacturers' recommendations.

Studies of the effectiveness of 22 different products for mouth washing, commonly available in department stores and pharmacies, were performed against 4 test strains using 2 contact times: 5 and 60 min. These products, according to manufacturer's recommendations, were undiluted while tested.

Study of the effectiveness of Mouthwash with fluoride, Elgydium, Paroplak, and Sensikin against 4 bacterial strains showed that they failed to achieve a 5 log reduction the number of viable bacteria, even after 1 h of contact time. The product 'Mouthwash with fluoride' was used as a control because the manufacturer has not declared any antibacterial activity.

A 5 log reduction in the number of bacterial cells for all tested strains after 5 min of contact time was proven for the following products: Aquafresh, Eludril, Listerine Freshmint, Listerine Cleanmint, Oral B Advantage Mouth Rinse, Denivit, Active Oral Care Extra Strength Original, Cepacol 'Antibacterial Mouthwash with Ceepryn', and Walgreens Fresh Breath Antiseptic Mouth Rinse.





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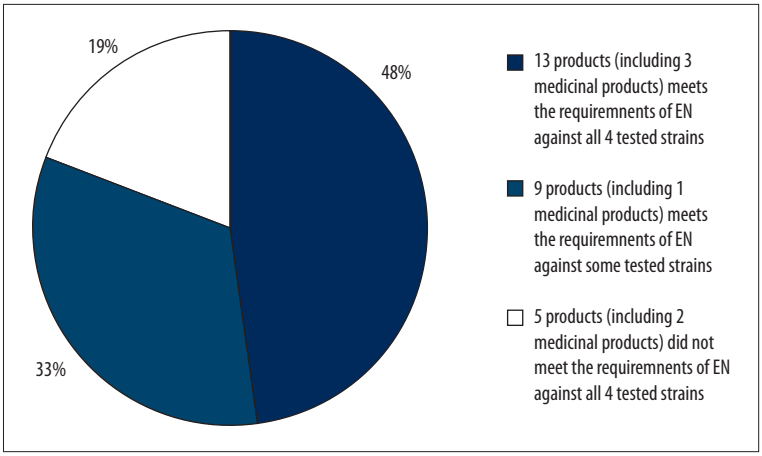


**Figure 1.** Examined products for rinsing and mouth disinfection currently on the Polish market. 5 min contact time. (n=27, including 6 medicinal products, 16 cosmetics and 5 other).

In the study of the effectiveness of the products Mouthwash antiseptic and Elmex, reduction in the number of *S. aureus* cells, required by the standard, was noticed after 60 min of contact time, and for the other tested strains after 5 min of contact time.

The evaluation of the efficacy of the Hascosept, Protefix Dental Antisept, and Oreksyd products showed bactericidal activity in accordance with PN-EN 1040 standard against the tested microorganisms after 5 min and after 60 min, only in the case of *E. hirae*.

Meridol and Lacalut Aktiv were effective against *E. coli* and *P. aeruginosa* after 5 min of contact time, but 60 min was required for *S. aureus* and *E. hirae*.

Studies of the bactericidal activity of Orthokin did not show enough efficacy against *E. hirae*, but the 5 log reduction in number of viable bacteria for the other 3 strains was achieved after just 5 min.

In the case of Pearl Drops, the effectiveness of the bactericidal activity against *P. aeruginosa* was demonstrated after 5 min; however, the action against the other tested strains did not meet with the standard.

Figure 1 illustrates the antibacterial effectiveness of the products used for mouth hygiene and disinfection. A total of 13 out of 27 products (48%), including 3 medicinal products, demonstrated bactericidal activity against all tested strains in accordance with the standard. Up to 19% of the products, including 2 medicinal products, did not demonstrate bactericidal activity against any of the tested strains according to PN-EN 1040. One-third of the tested products caused the reduction of bacterial cells required by PN-EN 1040, but only in 1–3 strains.

## Discussion

The information provided by manufacturers on the packaging or in the leaflets of products for mouthwash and disinfection is important for consumers. The manufacturers of antiseptic products should provide data on the concentration, duration of application, and scope of the antimicrobial activity.

Our study investigated whether the common, commercially available medicinal products and cosmetics meet the current, basic bactericidal requirements of the PN-EN 1040 standard for chemical disinfectants and antiseptics.

It was assumed that a product intended for use as an oral antiseptic is effective when the reduction of bacterial cells is on the level of at least 5 log after 5 min of contact time, which corresponds with the standard and is actually a reasonable mouth washing time. The mouth washing time can be shorter, however, when washing with water is not done, as a substantial amount of the product remains on the mouth mucosa for some time and allows the antibacterial effect to last longer. Antibacterial activity was also studied after 60 min of contact time, as it is the longest time specified by the standard PN-EN 1040 for disinfectants and antiseptics.

International, standardized test methods for evaluation of antimicrobial activity of preparations for oral cavity disinfection do not exist. Thus, researchers generally use different bacterial strains (ie, clinical isolates) and different assay conditions to evaluate antibacterial activity of mouth disinfectants. Results obtained this way are impossible to compare.

The European Committee for Standardization (CEN) has created several European Standards for evaluation of antimicrobial activity of chemical disinfectants and antiseptics. Standards concerning the test methods of Phase 1, for evaluation of basic antibacterial and antifungal activity (EN 1040, EN 1275), are aimed at general applications. There is no differentiation between antimicrobial requirements for antiseptic preparations applied on living tissue (skin and mucosa) and disinfectants used in order to diminish the microbial count on surfaces and instruments (medical devices). The CEN standards relate to





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only a limited range of microbial species and a limited number of standard strains. These have been chosen as representative, taking into consideration their relative resistance and their relevance to practical use. The CEN plans to evaluate the Phase 3 tests performed under practical conditions, with usage of clinical isolates; however, no single standard is available and only standard strains are recommended in the EN.

The oral microbes colonizing the surfaces of oral cavity mucosa, teeth, and tongue, as well as present in saliva, were not included in the study because of the diversity of strains. There are hundreds of species of oral bacteria, some of them pathogenic. These bacteria may be even more susceptible to the analyzed antiseptic preparations than standard strains recommended in the EN. However, CEN's idea was to use resistant rather than susceptible strains in tests. The incorporation of oral microbes in tests may be more appropriate, taking into consideration the possibility of control of oral health problem by use of antiseptics, but the aim of our study was to use the international, standardized, CEN method dedicated for evaluation of antibacterial activity of antiseptics, and to compare this activity of the products for mouth washing and oral disinfection present on the Polish market.

This study involved 2 groups of products: 1) medicinal products containing herbal extracts used against infections and inflammation of the mucous membranes of the mouth and 2) popular, widely available, liquids (rinses) for oral hygiene.

Hayes et al. [3] claimed that the active ingredients (essential oils) are used in medicinal products and antiseptics because of their antimicrobial activity and because of a lack of resistance mechanisms to these substances. Antimicrobial activity of essential oils depends on their chemical composition and it is related to plant species, vegetative parts of the plant used, and phase of plant development, as well as soil and climate conditions. Shapiro et al. [4] showed a high efficacy of peppermint, sage, and tea tree oils, thymol and eugenol, against bacteria colonization of the mouth. Adams and Kunz [5] proved that essential oils, due to their lipophilic properties, damaged the cell walls and cytoplasmic membranes of bacteria and fungi, leading to the leakage of cytoplasm. These oils inhibited the synthesis of DNA, RNA, proteins, and polysaccharides, as well as enzymatic activity. Antimicrobial activity of essential oils is determined by the activity of individual components. The synergistic effect of essential oils components that influence biocidal action is often observed.

Kalemba [6] studied essential oils derived from plants in our climate zone, such as thyme, rosemary, yarrow, mint, sage, and clove. He showed that each of these essential oils possesses antimicrobial activity against at least 1 organism, and the efficacy increases with oil concentration. Takarada et al. [7] studied the susceptibility of pathogenic bacteria to various antiseptics used in the mouth, and proved that tea tree and eucalyptus oils were biocidal to bacteria that cause paradontosis and dental caries. It was shown that a 0.2% solution of essential oils kills *Porphyromonas gingivalis* and *Streptococcus mutans* after 30 s. Hammer et al. [8] showed that tea tree oil inhibits the growth of other bacteria present in the mouth. The most sensitive bacteria belong to the genera *Porphyromonas* and *Prevotella*, and the least sensitive were *Streptococcus* and *Lactobacillus*. Kedzia's study [9] demonstrated the efficacy of Dentosept herbal product against anaerobic bacteria that cause periodontal disease. This product contains extracts of herbs such as chamomile, oak bark, sage, arnica, sweet flag, mint, and thyme. Kedzia's studies [9] showed high activity of this product against all tested microorganisms. Strains of *Bacteroides* and *Porphyromonas* genera showed the greatest sensitivity. There is an important fact concerning the obtained results – the author evaluated the smallest concentration (MIC) of Dentosept that inhibited the growth of microorganisms and interpreted the results after 48 h of culture. This method is used in studying antibiotic activity, but it should not be used in the case of antiseptics because of the increased length of contact time. In this publication, activity of the Dentosept A product (made by the same manufacturer) against aerobic bacteria was studied (the product contains the same set of plant extracts as Dentosept, but in lower concentrations). In this study, the undiluted product demonstrated activity complying with the standard. The results described by Kedzia [9], and obtained by this study, showed that Dentosept A may be used for mouth prophylaxis and the treatment of oral infections caused by aerobic and anaerobic bacteria. The Salviae, Salviasept, and Stomatosol products, used in significantly higher concentrations than recommended by the manufacturer, showed bactericidal activity against the 4 referenced strains tested in accordance with the standard.

After 5 min of contact time, the Salviae product in 40% concentration (approximately 28% ethanol) and the Salviasept product in 20% concentration (approximately 10% ethanol) showed reduction of bacteria as required by the standard. The Stomatosol product in 20% concentration (approximately 14% ethanol) was active after only 60 min of contact time. Control tests showed that such ethanol concentrations did not exhibit any antimicrobial activity. This study suggests the use of higher concentrations of plant extracts in products for mouth antisepsis than is recommended by the manufacturers.

The second group of products tested in this study was commercially available mouthwashes with antibacterial activity declared by the manufacturers. Two out of 23 of these products are included in the Register of Medicinal Products in Poland, and 16 out of 23 are included in the Central Register of Cosmetics. Many of them contain alcohol or chlorhexidine, or both. Bocian





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and Tyski [10] reported that solutions of ethanol in 60–70% concentrations were the most effective against *S. aureus* in dry or humid environments. Rotter's research [11] showed that survival of spores in ethanol depends on the species of bacteria and the concentration of alcohol. Spores of *Bacillus subtilis* remain viable in 95% ethanol for many years. However, spores of anaerobic bacteria survived in 10% ethanol for more than 10 months, but only as long as 4 weeks in 80% ethanol.

In the present study, we demonstrated that Hascosept containing ethanol and benzydamine hydrochloride has antibacterial properties towards ¾ of tested microbial strains after 5 min contact time, but in the case of *E. hirae,* 60 min of contact time was required. Other combinations of active substances are also used in mouth washing products, like the combination of 0.12% chlorhexidine with 0.05% acetyl-pyridine chloride (PerioAid), or alcohol with 0.2% chlorhexidine (Corsodyl).

Van Strydonk et al. [12] demonstrated the comparable efficacy of both preparations in reducing dental plaque. The products Aquafresh, Cepacol and Active Oral Care Extra Strength Original were proven to act as good oral antiseptics. Andre et al. [13] demonstrated bactericidal activity of Cepacol towards *S. mutans* grown on dentures as a biofilm. The product named Pearl Drops tested in this study did not show bactericidal activity in accordance with the standard. Due to the fact that manufacturers of the tested rinses did not provide quantitative information about the composition, it can be assumed that the ineffectiveness of Pearl Drops is caused by a concentration of active substances that is too low. The enrichment of an alcohol antiseptic product with chlorhexidine caused longer efficacy. As reported by Bocian and Tyski [14,15], antiseptic chlorhexidine gluconate is often used in dentistry and oral hygiene. Apart from antimicrobial properties and the effect of prolonged activity, it also exhibits high affinity for the mucosa and the ability to inhibit the formation of dental plaque. At the same time, however, it is characterized as not very harmful. In the present study, 4 washing mixtures containing both alcohol and chlorhexidine were investigated.

The products Eludril and Denivit were found to be effective against tested microorganisms after 5 min. The product Protefix Dental Antisept required 60 min of contact time for 1 of the strains; this is a very long time and, therefore, not practical for an antiseptic mouth rinse. The product Paroplak has no adequate biocidal activity against any tested bacteria. Quirynem [16], who studied the effectiveness of chlorhexidine at 0.12% and 0.2% concentrations, achieved comparable reduction in the number of bacteria: 78% and 89%, respectively. Despite the best results being observed for concentrations of 0.2%, the obtained difference was not statistically significant. The author suggests the possibility of reducing chlorhexidine concentrations without a significantly negative influence on therapeutic effect. Clinical studies performed with 0.12% chlorhexidine solution showed an 81–90% reduction of tongue microflora and 89–95% reduction of saliva microorganisms [17,18]. The products for mouth washing containing 0.2% chlorhexidine are recommended for the control of dental plaque and for the support of periodontitis treatment [19,20]. Gos-Slomka [21] conducted research at the Centre for Pediatric Pulmonology in Karpacz and showed a significant efficacy of the product Corsodyl (chlorhexidine gluconate 0.2%) in reducing the amount of dental plaque and reducing gingivitis. This decrease was noticeable most clearly for children aged 8–12 years, after the first 3 days of washing. A significant improvement in gingivitis was obtained both by decline in the amount of dental plaque as a major etiological agent of gingivitis and by the antibacterial activity of Corsodyl.

Limited studies of antimicrobial activity according to custom methods on products containing chlorhexidine, benzydamine, salvia, and gel with polyvinylpyrrolidone-sodium hyaluronate were carried out using microbial strains colonizing the oral cavity – *Enterococcus faecalis* and *Candida* sp. Reduced incidence of such colonization took place when polyvinylpyrrolidone-sodium hyaluronate gel was applied [22].

Luc et al. [23] studied biocidal activity of Corsodyl using the dilution-neutralization method. They observed efficacy against *S. mutans, Lactobacillus acidophilus, Fusobacterium nucleatum, Prevotella intermedia, Actinobacillus actinomycetemcomitans,* and *Candida albicans* after 1 min of contact time. Chen et al. [24] also demonstrated *in vitro* activity of the products Corsodyl and Listerine on *S. mutans* biofilm. Listerine showed stronger bactericidal effect, but had less bacterial inhibitory effects than Corsodyl. Our studies showed that Corsodyl was effective against *S. aureus, E. hirae, E. coli,* and *P. aeruginosa*. This was confirmed by the results described in the literature regarding the antimicrobial activity of this product and chlorhexidine at the concentration of 0.2%. Luc et al. [23] investigated the antimicrobial activity of the products Eludril (chlorhexidine gluconate and alcohol), Meridol (amine fluoride) and Lacalut (chlorhexidine gluconate) using the dilution-neutralization method after 1 min of contact time against *S. mutans, L. acidophilus, F. nucleatum, P. intermedia, L. actinomycetemcomitans,* and *C. albicans*. They observed the efficacy of Eludril at dilutions of 1:2 and 1:3 against all tested strains. Our studies showed a strong bactericidal activity of undiluted product against tested bacterial strains after 5 min of contact time. Luc et al. [23] showed that the product Meridol was active after 1 min of contact time only against *S. mutans* and *F. nucleatum*. The results obtained i our study demonstrated that Meridol met the requirements of the PN-EN 1040 standard after 5 min against strains of *E. coli* and *P. aeruginosa*, but in the case of *S. aureus* and *E. hirae* the product was equally effective only after 60 min.





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This observation confirms that this product has a bactericidal activity only against certain microorganisms. The studies of Luc et al. [23] described earlier showed that the product Lacalut was not an effective antiseptic. The results in this publications show that the rinse was effective against *S. aureus* and *E. hirae* after only 5 min and effective against all tested reference strains after 60 min. The difference in the efficacy Lacalut described in the above-mentioned study and our study may be caused by longer contact time and use of other microorganisms.

## Conclusions

1. Investigated plant products with a declared antimicrobial activity registered as medicinal products, in most cases, did not exhibit the antibacterial activity required by the PN-EN 1040 standard at the recommended concentrations.
2. Only some mouth washing products exhibit antimicrobial activity required by the standards.
3. The information on the packaging or the leaflets for antiseptic products should be corrected by the manufacturers to include accurate information on antimicrobial activity.

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